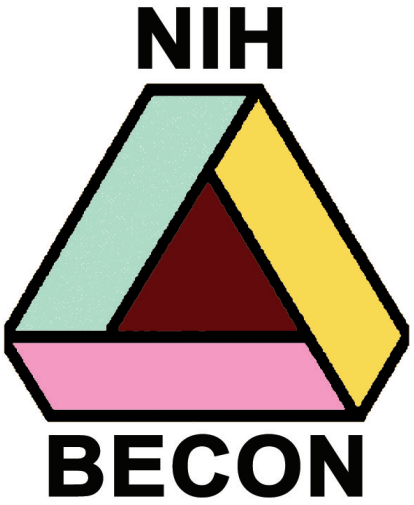
Bioengineering Research Partnerships
Third Annual Grantee Meeting



June 24-25, 2003

Bioengineering Consortium
National Institutes of Health
Bethesda, Maryland

Bioengineering Consortium

National Institutes of Health
Bethesda, MD 20892

Welcome to the Third Annual Bioengineering Research Partnership Grantee Meeting.

This February marked the sixth anniversary of the Bioengineering Consortium (BECON) which provides a focus for biomedical engineering research and training activities at the National Institutes of Health (NIH). Active participation by all NIH centers, institutes, and offices and other Federal agencies has facilitated the realization of substantial benefits from the application of engineering, physical, and computational science principles and techniques to address problems in biology and medicine. The importance of this field is reflected by the steady increase in total annual funding for bioengineering research over the past six years and the establishment of a National Institute for Biomedical Imaging and Bioengineering at the NIH.

To facilitate the development of the field of bioengineering, the BECON has coordinated trans-NIH initiatives aimed at encouraging and supporting multi-disciplinary and integrative approaches to biomedical research and training. One of the most successful and visible of these research initiatives is the Bioengineering Research Partnership (BRP) Program which was first announced in October 1999. The partnerships that have developed in response to this program are examples of the types of collaboration between the biomedical sciences and allied technical disciplines that can provide significant advances for improving human health. To date, over 100 BRP awards have been made for a total investment of over $318 million by sixteen NIH research institutes and centers.

This meeting is the third time that the BRP grantees, BECON members, and NIH institute/center representatives will gather to discuss the research projects, related issues, and the program in general. Your perspectives and suggestions concerning partnership experience and management, program efficacy, bioengineering research and training needs and directions, and the format for future BRP grantee meetings are solicited. Also, please take this opportunity to meet with your NIH institute/center representative to discuss progress and concerns for your project.

I hope that the BRP Grantee Meeting is valuable and enjoyable to you. All the BECON members and NIH program staff look forward to your participation and comments.

Dr. Jeff Schloss, Chair
Bioengineering Consortium

BIOENGINEERING RESEARCH PARTNERSHIP
THIRD ANNUAL GRANTEE MEETING
June 24-25, 2003
Bethesda, MD

AGENDA

Tuesday, June 24, 2003

2:00 PM **Welcome and Logistics (Grand Ballroom)**
Richard Swaja and Jeff Schloss

2:15 PM **NIH Topics of Interest (Grand Ballroom)**
Current Issues at the NIH - *Regina White (NIH/OER)*
Technology Transfer: Sharing of Research Tools and Invention Reporting - *George Stone (NIH/OER)*
Review of Bioengineering Research Applications - *Donald Schneider and Anita Miller Sostek (CSR)*

3:30 PM **Break**

4:00 PM **BRP Grantee Presentations I**
Group 1 - *Moderator: Christine Kelley (NIBIB)* - Grand Ballroom
Group 2 - *Moderator: Maren Laughlin (NIDDK)* - Bethesda/Potomac
Group 3 - *Moderator: Houston Baker (NCI)* - Rockville/Chevy Chase

5:30 PM **Group Discussion (Grand Ballroom)**

6:00 PM **Networking Session (Grand Ballroom)**

Wednesday, June 25, 2003

7:00 AM **Continental Breakfast (Grand Ballroom Foyer)**

8:00 AM **Orientation (Grand Ballroom)**
Richard Swaja

8:15 AM **BRP Grantee Presentations II**
Group 4 - *Moderator: Jeff Schloss (NHGRI)* - Grand Ballroom
Group 5 - *Moderator: Lynn Luethke (NIDCD)* - Kenwood/Montrose
Group 6 - *Moderator: Martha Lundberg (NHLBI)* - Montgomery/Democracy

9:45 AM **Break**

10:15 AM **Group Discussion (Grand Ballroom)**

12:00 PM **Adjourn**

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute (NHLBI)

PROJECT TITLE: Optimizing Heart and Brain Cooling during Cardiac Arrest

ABSTRACT:
Cardiac arrest currently has a less than 5% survival rate and hypothermia may be an important therapy to improve this poor outcome. The poor survival is due to the brief time paramedics have to restart blood flow to ischemia-sensitive organs such as the heart and brain. The recent NIH PULSE Conference identified cooling as one of the "most promising" areas of research to improve cardiac arrest survival. While techniques such as cardiopulmonary bypass and aortic flush can rapidly induce hypothermia, the complexity of their initial setup makes them less practical for cooling in the field. Therefore, designing a rapid cooling system usable in the field is an ideal focus for a bioengineering research partnership in which engineers and clinicians combine efforts. We hypothesize that cooling after cardiac arrest can be rapid (despite the low blood flow conditions of CPR) and practical for paramedics under field conditions, and will improve survival. We propose a novel cooling system to be developed in a swine model of cardiac arrest, with the ultimate goal of human application by paramedics. Our partnership of physicians, biologists, and engineers at the University of Chicago and Argonne National Laboratory has modified for medical application ice slurry technology originally developed for cooling large buildings. These microparticle ice slurries flow like liquids, but have up to 8 times the cooling capacity of a similar volume of cold liquid (0-4C) without ice, facilitating heat transfer with lower volumes and flow. For this project, we have developed two novel ice-particle slurries: (a) a saline-based ice slurry (saline slurry) that can be used through existing intravenous catheters or pumped into the stomach and (b) a perfluorocarbon-based ice slurry (PFC slurry) for endotracheal instillation into the lungs. In preliminary studies, these prototype ice slurries used in tandem have achieved remarkable cooling rates of approximately 0.5 C/min within the heart and brain during cardiac arrest with chest compressions – and have the potential to cool even faster with improved formulations. Using prototype PFC slurry as a coolant in the lungs of normal animals, some toxicity resulted (consistent with other reports of PFC toxicity), but animals survived unassisted for 48 hours with normal oxygenation with improving respiratory function, and had only mild pathological changes on histology. Thus our preliminary data suggests developing an optimal cooling method with minimal adverse effects to be a realistic goal. This hypothermia system represents a new option for induction of intra-arrest low flow hypothermia to be rapidly performed by paramedics in the field. To further promote the development of this project, a multi-center international advisory board of noted cardiac arrest experts will assist the Partnership. With a cooling system engineered to surmount this heat-transfer challenge, multi-center animal studies could quickly lay the scientific foundation for implementing what could become a new and effective cardiac arrest treatment by paramedics in the field. The development of cooling therapies may also be useful in the treatment of myocardial infarction, stroke, brain injury, as well as in comatose survivors of cardiac arrest.

STATUS OF RESEARCH AND PARTNERSHIP:
Cardiac arrest currently has a less than 5% survival rate and hypothermia may be an important therapy to improve this poor outcome. The poor survival is due to the brief time paramedics have to restart blood flow to ischemia-sensitive organs such as the heart and brain. The recent NIH PULSE Conference identified cooling as one of the "most promising" areas of research to improve cardiac arrest survival. While techniques such as cardiopulmonary bypass and aortic flush can rapidly induce hypothermia, the complexity of their initial setup makes them less practical for cooling in the field. Therefore, designing a rapid cooling system usable in the field is an ideal focus for a bioengineering research partnership in which engineers and clinicians combine efforts. We hypothesize that cooling after cardiac arrest can be rapid (despite the low blood flow conditions of CPR) and practical for paramedics under field conditions, and will improve survival. We propose a novel cooling system to be developed in a swine model of cardiac arrest, with the ultimate goal of human application by paramedics. Our partnership of physicians, biologists, and engineers at the University of Chicago and Argonne National Laboratory has modified for medical application ice slurry technology originally developed for cooling large buildings. These microparticle ice slurries flow like liquids, but have up to 8 times the cooling capacity of a similar volume of cold liquid (0-4C) without ice, facilitating heat transfer with lower volumes and flow. For this project, we have developed two novel ice-particle slurries: (a) a saline-based ice slurry (saline slurry) that can be used through existing intravenous catheters or pumped into the stomach and (b) a perfluorocarbon-based ice slurry (PFC slurry) for endotracheal instillation into the lungs. In preliminary studies, these prototype ice slurries used in tandem have achieved remarkable cooling rates of approximately 0.5 C/min within the heart and brain during cardiac arrest with chest compressions – and have the potential to cool even faster with improved formulations. Using prototype PFC slurry as a coolant in the lungs of normal animals, some toxicity resulted (consistent with other reports of PFC toxicity), but animals survived unassisted for 48 hours with normal oxygenation with improving respiratory function, and had only mild pathological changes on histology. Thus our preliminary data suggests developing an optimal cooling method with minimal adverse effects to be a realistic goal. This hypothermia system represents a new option for induction of intra-arrest low flow hypothermia to be rapidly performed by paramedics in the field. To further promote the development of this project, a multi-center international advisory board of noted cardiac arrest experts will assist the Partnership. With a cooling system engineered to surmount this heat-transfer challenge, multi-center animal studies could quickly lay the scientific foundation for implementing what could become a new and effective cardiac arrest treatment by paramedics in the field. The development of cooling therapies may also be useful in the treatment of myocardial infarction, stroke, brain injury, as well as in comatose survivors of cardiac arrest.

ISSUES:
We intend to make advances in slurry generation by increasing to 60% ice slurry, by gaining more consistency in slurry fluidity, and by optimizing rapid "on-site" slurry generation methods. In the animal laboratory, we intend to expand our capability with improved hemodynamic measurements via the acquisition of intra-cardiac pressure-volume loop technology and by gaining experience with fluorescent microspheres. We will also continue to pursue the experiments outlined that compare the cooling effectiveness with differing methods of administration of slurry (IV, pulmonary, and GI routes separately and in combination). We will continue to explore the possible adverse effects of slurry under all these routes of administration. Important Partnership issues include: (a) Continuing to optimize communications with the use of a "virtual laboratory" environment (the Access Grid) in an effort to cope with the physical separation between University of Chicago and Argonne National Laboratory. (b) Developing and communicating clear research expectations for different team members at different sites. (c) Maintaining a common "team vision" while the institutions of origin have widely divergent missions.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB) and National Institute of Neurological Disorders and Stroke (NINDS)

PROJECT TITLE: Probing Single-Molecule Neuron-Ligand Pathfinding

ABSTRACT:
Our BRP program is aimed ultimately at the development of a new biomaterial that can be implanted, injected or otherwise delivered to sites of damage or injury to the central nervous system for the restoration of lost function and the regrowth of damaged neurons. The program addresses a pressing health need affecting millions of people yearly with an estimated cost of several billion dollars yearly in the US. Our program aims to develop a fundamental understanding of the molecular-level details that govern neurite outgrowth, rate, directionality, connectivity and adaptability as these properties are modulated by cell age and history, surface ligand coverage and composition, ligand orientation and flexibility, and ligand density gradients will help to guide the development of these next-generation products and treatments.

STATUS OF RESEARCH AND PARTNERSHIP:
Year-1 of this project was focused on the production and detailed characterization of well defined substrates and their use in quantitative measurements of neurite outgrowth. Excellent progress was made toward this aim, with the following high points: Surfaces and atomic force microscope (AFM) tips have been made and characterized by x-ray photoelectron spectroscopy (XPS), AFM and time-of-flight secondary ion mass spectrometry (TOF-SIMS). These surfaces and tips present covalently attached fibronectin protein (FN) and short RGD peptides from the binding domain of FN. Technical hurdles of shipping live neurons have been overcome at both ends (Utah and Delaware), as has the shipping of sterile and biologically active surfaces in both directions. Construction and standardization of the fluorescence correlation spectroscopy (FCS) apparatus has been completed in the Hlady labs, and the first data on live neurons has been obtained. Considerable purchase and build-up of new instrumentation have been completed, even amid the move of the Beebe Labs from Utah to Delaware.

Year-2 of this project has been focused on the initial uses of the carefully characterized substrates for the growth of neurons, continued refinement of new surface chemistries for the "bioactive" attachment of ligands, the patterning and spatially resolved characterization of these chemistries on the micron scale, and the extension of these chemistries to AFM tips. The first direct AFM force measurements are now being made as we approach the end of Year-2. Three publications acknowledging support from this grant are in print, and two more are within two weeks of submission. Additional scientific details are given below.

Neuronal growth on patterned substrates. The interaction of rat dorsal root ganglia (DRG) neurons was examined on surfaces patterned with micron-sized squares and lines of varying width, presenting fibronectin interrupted with lanes of non-adhesive comb polymer (see new publication featured on the cover of Advanced Materials). We verified the utility of the substrate to spatially control cell attachment and growth for future studies outlined in the original proposal. DRGs were cultured for 24 hours on the

substrates, fixed in paraformaldehyde and processed for immunofluorescence. Double-labeling with antisera for neuronal-specific βIII-tubulin and fibronectin showed neuronal attachment specifically restricted to regions containing fibronectin. Neuronal outgrowth was successful and the neurons demonstrated good elaboration and exploration of the FN-covered regions, with avoidance of the comb polymer-covered regions.

FCS Experiments. One of the novel experimental approaches being pioneered for live-cell applications in this BRP by the Hlady group is fluorescence correlation spectroscopy (FCS). The technique allows us to make observations of the dynamics of neuron-ligand interactions by observing the time-dependent fluctuations of fluorescence signals at the single-receptor level. A significant verification experiment was recently performed. A membrane-specific dye [1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate, "DiI"] was placed in nanomolar concentrations in the bath of fixed dorsal root ganglion cells. Analysis of the data was performed according to published work, and the extracted variables were comparable to published results in other systems. It is clear from these first results that "DiI" in cell membranes exhibits non-Brownian motion as was argued in previous work. These experiments provided a foundation on which we are now probing membrane integrin receptor dynamics on spatial and temporal scales never before attempted.

Status of Partnership. Despite possible problems raised in reviews about the ability to successfully ship live neurons over such long distances, we have been able to do so in several different ways. The PI has been able to make numerous visits to the Utah site for research progress meetings and for the development of new capabilities at Utah, especially for surface patterning and comb polymer chemistry. The team at Delaware has also become adept at limited tissue culture experiments with live cells, and fluorescence microscopy measurements, both of which were unknown capabilities as the program began.

ISSUES:
This program began, coincidentally, at the same time as the move of the PI from Utah to Delaware. Prior to this, during proposal review, direct costs were approved on the basis of a single grant to one institution. Following the move of the PI, it was necessary to establish a large subcontract to the University of Utah, where two of the three PIs are located. The indirect costs of the Utah subcontract are counted in the project's direct costs, as is customary. However, these direct costs were held fixed at their original values prior to the move. In effect, the available budget for scientific expenditures was therefore reduced by 30%. These shortfalls were absorbed in Year-1 and Year-2, limiting the planned hiring of personnel and several expenditures. We were advised by institute personnel to submit a request for restoration of funds to their original approved levels following the submission of our latest major results. Despite these unforeseen problems, we have been able to make excellent progress in a new project after less than two years.

No animal procedures are being performed or are proposed to be performed at Delaware, and all animal procedures are being performed and are proposed t be performed at Utah. In Year-1, we were required to submit only Utah's approved IACUC animal-use protocol. When our grant was transferred in majority from NINDS to NIBIB in Year-2, we were asked to submit an approved IACUC from Delaware where none was in place. This caused unexpected delays and a freeze on some expenditures for animal-related work at Utah during Year-2. There were also additional delays in Delaware's establishment of a timely subcontract to Utah in Year-2, caused apparently by a typographical error in the grant's number at the institute at the time of institute changeover. We are optimistic that these delays are now fully solved and will not impact our program negatively. We are enthusiastic about a competing continuation for this program, planned for a 1 November submission (shortly after the start of Year-3) and requesting a 5-year program with proper budgeting for the subcontract.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB)

PROJECT TITLE: An Integrated Platform for Chemical Analysis of Live Cells

ABSTRACT:
The overall aim of this partnership is to design, build and test an integrated optical and microfluidics system that will enable the performance of novel biochemical assays in single, living cells. The specific aims of the research are (1) to develop a laser microscope platform for single cell manipulation and analysis, (2) to develop a multipurpose, modular microfluidics chip for single cell analyses, and (3) to develop a broad range of analytes for cell assays. The development process involves basic studies of the physical mechanisms of laser interactions with cells and polymeric materials used to manufacture the chips, basic engineering of polymer-based microfluidic devices, integration of the microfluidic devices and microscope platform, and further development of novel biochemical assays to be performed with the integrated system.

STATUS OF RESEARCH AND PARTNERSHIP:
In the fourth year of this project, significant progress continues to be made. Progress has been made in areas of laser microbeam interactions, MEMS integration for on-chip cell lysis, and instrument control.

One important focus in the last year has been to develop a system to visualize the dynamics of laser microbeam interactions with cells when applied for cell lysis and optoporation. These dynamics are critical to understanding the temperatures and pressures attained and the hydrodynamics involved in these processes and their implications for developing a MEMS device using this technology. The imaging system we have developed allows us to do time-resolved imaging on cell samples from sub-nanosecond to 100's of microseconds with a spatial resolution of a few microns using a Nd:YAG laser. Studying the dynamics has allowed us to calculate shock wave speeds and peak pressures and measure cavitation bubble sizes, oscillation times and bubble energies and time-scales of the process. Current work concentrates on producing a physical model of the breakdown process which would allow us to correlate the physical and biological effects. Additional efforts include the study of micro-channel and micro-cavity designs to optimize the lysis of cells and subsequent removal of lysate in polymer chips. Computer imaging technology and robotic control instrumentation has advanced such that a laser microscope can be controlled from a computer terminal over an internet connection.

We have developed strategies using ultraviolet light to polymerize mixed monomer solutions onto the surface of a poly(dimethylsiloxane) (PDMS) microfluidic devices. By including monomers with different chemical properties, electrophoretic separations were optimized for a test set of analytes. The properties of surfaces grafted with a single neutral monomer, a neutral and a negative monomer, or a neutral, negative, and cross-linking monomer were assessed. This has helped to solve one of the most limiting problems in polymer microfluidic chips—control of the wall surface chemistry. High quality separations were achieved in PDMS microfluidic channels with cross-linked coatings. The separation efficiency for biologically relevant peptides (kinase substrates) on these surfaces was as high as 18,600 theoretical plates in a 2.5 cm channel, and separations between two different peptides occurred in as little as 400 ms after injection. The simultaneous separation of five kinase and phosphatase substrates was also demonstrated. By carefully selecting mixtures of monomers with the appropriate properties, it may be possible to tailor the surface of PDMS for a large number of different electrophoretic separations. Biochemical assays continue to be developed for use in the microfluidic chips. Fluorescent labeling and deprotection protocols for the preparation of enzyme substrates to be used in the biochemical assays are being optimized and put into practice.

Finally, the commercialization of the single cell analysis system has progressed both through the filing of UC intellectual property disclosures, provisional patent applications, and licensing of technology. The chemical surface treatment has been demonstrated to be of great utility to polymer microfluidic chips as well as to other biomedical applications such as implantable devices. UC is currently in negotiation with two companies to license relevant portions of the surface grafting technology.

The active collaboration of the multidisciplinary researchers has been a significant strength in our research effort. Almost every aspect of the above-described work has involved the close interactions among members of the partnership. Concrete examples of this interactive relationship are shared postdoctoral fellows between the investigators, collaborative supervision of graduate students in multiple laboratories, regular attendance of investigators at the lab meetings of fellow investigators, and formal meetings of all the investigators, postdoctoral fellows and students involved in the project. The format of these combined meetings consists of a formal presentation by an individual investigator of work relevant to the partnership followed by a general discussion of issues involved with the research. Attesting to the success of this research program are papers co-authored by various investigators within the partnership.

ISSUES:
None.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB)

PROJECT TITLE: Breast CT Scanner for Earlier Cancer Detection

ABSTRACT:
Breast cancer is a disease with high incidence in the U.S. and elsewhere, and population-level methods of fighting this disease are aimed primarily on screening, using mammography for early detection. We hypothesize that breast CT may be able to routinely outperform mammography in breast cancer detection. The radiation dose of breast CT performed at 80 kVp was found in detailed studies to be comparable to that of mammography. We have teamed with scientists from around the country to design, build, and test a CT scanner designed to image the breast. A team comprised of medical physicists, physicians, mechanical and electrical engineers, and breast cancer advocates will collaborate on the design of the breast CT scanner. Cone beam flat panel technology will be used to produce a scanner capable of 10 second breast scanning, and the scanner development will also include a breast immobilization system, a breast CT table, a fast reconstruction computer, and a computer workstation customized for efficient viewing breast CT images.
The scanner will be built, tested, and optimized at UC Davis over a period of 3 years involving 9 specific aims. After the breast CT scanner is tested in a brief phase I trial (2 specific aims), it will be moved to the breast imaging clinic for a phase II trial where approximately 120 women will be imaged (4 specific aims). This phase II trial will evaluate the efficacy of breast CT for the early detection of breast cancer in a group of women likely to have breast cancer (BIRADS 4 & 5). Additionally, the breast image data will be studied for its utility in automating the analysis of the normal breast architecture, and for computerized cancer detection. In year 5 of the proposed research, two specific aims utilize the breast CT data and corresponding mammography images (on ~240 breasts) to evaluate the ideal observer performance and human (mammographer) detection performance attributes of the breast CT scanner.
In this project, the potential of breast CT will be evaluated both qualitatively and quantitatively. If breast CT lives up to its enormous potential based on initial imaging, breast cancer would be detectable far before metastases occurs – for example, a 3 mm tumor contains only 2% of the cell count of an 11 mm lesion, and a 5 mm lesion contains only 9% of the cell count. Based on a 100 day volume doubling time, detection of a 5 mm lesion would lead to 0.93 year earlier detection, and routine detection of 3 mm lesions would result in 1.5 year earlier detection over mammography. Surgical removal of early cancers will effectively result in cure for the majority of women screened using this technology. While breast CT would probably improve cancer detection in all women, some women may have risk factors (dense breasts, genetic markers, etc.)

that particularly warrant screening using breast CT. The Phase II trial will shed more light on this issue.

STATUS OF RESEARCH AND PARTNERSHIP:
The partnership is alive and well. Varian Imaging Systems is slated to deliver a high frame rate panel by the end of 2003. Duke University is beginning their study of Bayesian analysis for noise suppression, after a late start due to contract office difficulties. The image display workstation has been built a UC San Diego and the software for this system is being developed. Although it would be accurate to characterize progress as “slower than we would have liked”, we are all looking forward to having this project come together through our combined efforts.

ISSUES:
The biggest issues or problems have really been administrative. UC Davis (base institution for this project) was going through major changes in the Grants and Contracts office at the time of this award, and the subcontracts were therefore late in getting finalized. It appears that similar problems have taken place at Duke’s Grants Office, and their subcontract paperwork has been held up substantially. Although the design of the breast CT scanner is proceeding well, the Monte Carlo evaluation of the system has taken longer than expected and this had delayed our finalizing specific aspects of the design. This in turn has delayed the fabrication of the scanner, and we are only now beginning to order the crucial hardware which are necessary for the construction of the scanner. This means that most of our equipment funds will need to roll over into the second year of the award.

We look forward to the opportunity to talk to other BRP investigators at this meeting, because the scope of this project (scientifically, geographically, and temporally) is larger than this PI has encountered before. Communication is a key aspect to the success of this project, and efficient but productive communication channels need to be better developed. We plan on learning a lot in Bethesda.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Neurological Disorders and Stroke (NINDS)

PROJECT TITLE: An organotypic model of traumatic brain injury

ABSTRACT:
The past decade has witnessed intense scientific activity to investigate molecular mechanisms of traumatic brain injury, driven by overwhelming evidence that neuroprotection by pharmacological inhibition of apoptosis has the potential to dramatically reduce the effects of brain trauma. Key requisite for the systematic investigation of neuroprotective agents is an accurately characterized, clinically relevant in vitro brain injury model. Despite this obvious need, the ability to deliver such defined, realistic trauma to specimens in vitro lags far behind the sophistication of molecular and biochemical assays used to measure the response. This Bioengineering Research Partnership brings together neurobiologists and bioengineering scientists to developed an in vitro brain injury model, which subjects organotypic brain cultures to angular acceleration-induced shear injury. In this model, organotypic brain cultures realistically model the in vivo apparent heterogeneous cell population in a three-dimensional cellular matrix, while angular acceleration-induced shear strain delivers a scalable, defined, and clinically relevant mechanical insult.

We hypothesize that our acceleration model of organotypic brain cultures can realistically reproduce traumatic brain injury, where the delivered shear strain magnitude can be quantified on a cellular level. Exercising our model, we will be able to determine cell type specific injury vulnerability. Furthermore, we will determine if caspase-8 and caspase-9 affect cell death following brain trauma.

To date, we complete a formal experimental characterization of our novel brain injury system, including assessment of the delivered angular acceleration magnitude and determination of the constitutive properties of the organotypic specimen (Aim 1). The resulting experimental source data will be directly applicable to formulate a realistic analytical model that allows computational simulation of the shear injury throughout the brain specimen for any point in time during the primary mechanical insult (Aim 2). Based on and concomitant to this rigorous system characterization, we will exercise the brain injury model to establish dose/response histories (Aim 3), and we will delineate the effects of hypoxic brain injury (Aim 4), secondary to the mechanical insult. Finally, we will employ our organotypic trauma model to determine the neuroprotective potential of caspase-8 and caspase-9 (Aim 5).

Upon successful completion, the results of this integrative research approach will yield a well-characterized, scalable, reproducible and clinically relevant brain injury model. Considering the vast interest in therapeutic interventions now under development aimed at inhibiting the

cascade of secondary effects of primarily mechanical brain injuries, our organotypic trauma model will directly address the rapidly increasing demand for a well characterized, experimental system to deliver a clinically relevant traumatic insult – and may prove crucial for the discovery of caspase-based neuroprotective mechanisms.

STATUS OF RESEARCH AND PARTNERSHIP:

Albeit this research partnership is still in FY01, we were able to assemble a cohesive, interdisciplinary research team that has achieved considerable progress in three out of five specific aims.

Specific Aim 1: System Characterization. An optimized 2nd generation brain injury device has been developed and characterized. This entirely re-designed system is more user-friendly and allows to control both, magnitude and duration of the acceleration-induced shear strain delivered to organotypic brain slice cultures. To date, we successfully characterized three key aspects of the system: 1) The acceleration history of the rotation stage, on which the organotypic brain slice culture is mounted. 2) The deformation of the brain slice during the acceleration impulse was captured and quantified with a custom time-lapse photographic set-up. This approach allowed detection of deformations smaller than 0.02 mm during a mono-phasic acceleration pulse of less than 3 ms duration. 3) To determine the rate-dependent constitutive properties of organotypic cultures, as well as the strength of culture-to-membrane interface, organotypic brain slices were subjected to a parametric series of dynamic shear tests.

Specific Aim 2: Computational Modeling: Computational simulation is required to predict the shear strain distribution within the organotypic culture, which ultimately is responsible for the cellular injury cascade. Implementing the previously derived physical measurements into a commercial Finite Element Analysis code, we initially simulated acceleration of the organotypic brain slice with ANSYS Multiphysics code, which allows for transient dynamic analysis. Most recently we advanced this model toward an explicit dynamic analysis in LS-DYNA software, an extension product of ANSYS.

Specific Aim 3: In collaboration with the Dow Neurological Laboratory, we exercised the second-generation injury device at selected operating parameters to establish the dose-response characteristics of organotypic brain slices to increasing levels of acceleration, using enzymatic and immunohistochemical assay techniques.

ISSUES:

The collaboration within the BRP has progressed smoothly. Formal weekly meetings between scientists in the fields of neurobiology (Dr. Simon's Group) and engineering (Dr. Bottlang's Group) ensure continuous exchange of results and coordination of future strategies. Dr. Rochefort of Oregon State University visited Portland at several occasions to oversee dynamic sheer testing of brain slices. Most recently, we were able to attract Dr. Lusardi from Dr. Meaney's group at the University of Pennsylvania to further expand our collaboration with leading researchers.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Diabetes and Digestive and Kidney Disease (NIDDK)

PROJECT TITLE: High Tc susceptometer for magnetic measure of body iron

ABSTRACT:
This Bioengineering Research Partnership combines bioengineering, basic science and clinical efforts in the design, development and clinical validation of a high-transition-temperature (high-TC; operating at 77 K) superconducting susceptometer for the direct, non-invasive measurement of hepatic iron stores in patients with iron overload from hereditary hemochromatosis, thalassemia major (Cooley's anemia), sickle cell disease, aplastic anemia, myelodysplasia and other disorders. Our laboratories originally proposed that storage iron (ferritin and hemosiderin) could be non-invasively assessed in vivo because of its paramagnetic properties. We subsequently developed low-transition-temperature (low-TC; operating at 4 K) superconducting quantum interference device (SQUID) biosusceptometry as a clinical method for the measurement of hepatic iron stores. Non-invasive magnetic measurements of hepatic storage iron in patients with iron overload are quantitatively equivalent to biochemical determinations on tissue obtained by biopsy but the cost and complexity of the low-TC instrument has restricted clinical adoption of the method. The low-TC susceptometer has three elements which utilize superconductivity: (i) the SQUID, (ii) the field coils that produce a localized steady magnetic field near the liver, and (iii) the detection coils and flux transformer. During the past year we have constructed a prototype high-TC susceptometer for clinical studies that uses (i) magnetoresistive sensors to replace the SQUID, (ii) a NdFeB (neodynium-iron-boron) permanent magnet providing a strong localized magnetic field to replace the fild coils, and (iii) detection coils and flux transformer fashioned from high-TC "tape" operating with liquid nitrogen as the coolant. We have successfully used the high-TC susceptometer in measurements of phantoms and are constructing a gantry support system to use the instrument in the first human studies later this summer. The development of an affordable, readily usable instrument for the non-invasive measurement of hepatic iron would be a major advance in the diagnosis and management of patients with iron overload that would find immediate and widespread clinical use both in the U.S. and worldwide.

STATUS OF RESEARCH AND PARTNERSHIP:
Active. Despite some difficulties in production of the Ion-Beam-Assisted Deposition [IBAD] superconducting tape, rapid progress continues to be made in manufacturing and assembling the custom electronic and cryogenic components for the first clinical prototype susceptometer.

ISSUES:
BRP review and evaluation criteria; patent procedures

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GRANTING NIH INSTITUTE/CENTER: National Institute of Arthritis and Musculoskeletal Diseases (NIAMS)

PROJECT TITLE: Nonlinear Computational Biomechanics of the Hip

ABSTRACT:
Disorders of the hip comprise a substantial fraction of current musculoskelatal disease burden. Complex nonlinear mechanical phenomena pervade many aspects of treatment of hip disease and injury, including total hip arthroplasty, intra-articular fractures, osteonecrosis, and developmental dysplasia. While bioengineering capabilities exist – in principle – to quantify key mechanical factors influencing treatment outcomes in these areas, contemporary clinical decision making still rests almost entirely on subjective empirical experience. This Bioengineering Research Partnership (BRP) brings together the capabilities of an experienced computational biomechanics research group, four senior orthopaedic hip surgeons, a veterinary research orthopaedist, and an industry-based materials scientist, in order to advance the state of the art in biomechanically-grounded management of disorders and injuries of the human hip. The central focus of the research Partnership lies in applying nonlinear finite element formulations to address as-yet-unquantified mechanical phenomena that are clinically recognized as being crucial to patient outcome. Building on previous and ongoing finite element work, new computational formulations will be developed to tackle nonlinearities currently limiting the accuracy of numerical simulations in five clinically important areas of hip surgery. The first two areas involve leading complications of total hip arthroplasty. First, as regards abrasive wear of polyethylene, we propose to incorporate local directionality of femoral head counterface motion in computing wear rates with a sliding-distance-coupled contact finite element formulation. Second, as regards dislocation, we propose to introduce soft tissue tethering into a large-displacement sliding contact model of resistance to dislocation. The third area involves intra-articular fractures of the acetabulum: estimating residual cartilage contact stress elevations accompanying attempts at surgical restoration of articular surface congruity. The fourth area involves osteonecrosis: computationally characterizing a new animal model (the emu) which unlike previous animal models progresses to human-like femoral head collapse, and using that model for in-vivo testing of computationally optimized placement of a novel head-preserving implant device. The fifth application area involves surgical management of developmental hip dysplasia: using novel mesh pre-processing techniques to quantify improvements of intra-articular contact stress achieved by pelvic osteotomies. This Partnership will bring together a critical mass of engineers and surgeons, to achieve clinically-grounded advances in nonlinear numerical simulations of surgery of the hip.

STATUS OF RESEARCH AND PARTNERSHIP:
On cross-over polyethylene wear (SA1), we are in the data harvest phase of our work with wear dependence on motion directionality relative to femoral head scratching. Our global finite element wear formulation has been extended to form the backbone of an R01 project on 3rd body acceleration of polyethylene wear. On dislocation (SA2), our work with capsule mechanical property measurement is complete, and we now have a working 3-D model of dislocations with full capsule representation, including wrap-around effects. Our voxel-based contact finite element formulation is fully operational. Besides our ongoing BRP work with acetabular fractures (SA3) and with developmental hip dysplasia (SA5), we have ported this formulation to the ankle, which has provided key enabling technology for our department's SCOR grant on post-traumatic arthritis. Our work with the emu model (SA4) has included developing a cryo-insult probe to create segmental lesions, a thermal finite element model to assess the distribution of osteolytic critical isotherms, and an image analysis routine automatically histologically quantify empty (dead) versus occupied (live) osteocyte lacuna, thus mapping the zone of osteonecrosis. Building blocks created for our emu structural finite element model have included cortical and cancellous bone material property measurements, joint contact force measurement, contact stress distribution measurement, and CT voxel-based meshing. The full three dimensional model is now undergoing parametric trials. Our relationship with the College of Veterinary Medicine at Iowa State University has deepened, and we now have an R01 grant to extend our development of the emu osteonecrosis model.

ISSUES:
None. This BRP grant has absolutely fulfilled our goal of allowing us to incubate new computational formulations for articular joint biomechanics, which in turn have provided a springboard for innovative applications involving unsolved clinical problems in orthopedic surgery of the hip, and now other joint as well.

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GRANTING NIH INSTITUTE/CENTER: National Institute on Drug Abuse (NIDA)

PROJECT TITLE: Dynamic Signal Processing Analyses of Neural Plasticity

ABSTRACT:
In response to PAR-02-010, Bioengineering Research Partnerships, we propose to form a research partnership between a statistician (Dr. Emery N. Brown of Massachusetts General Hospital, Partnership Director), two neuroscience experimentalists (Dr. Matthew A. Wilson of the Massachusetts Institute of Technology and Dr. Wendy Suzuki of New York University) and a control engineer (Dr. Victor Solo of the University of New South Wales) to develop a systems engineering approach to understanding neural plasticity. The area of bioengineering research will be the development of neural signal processing algorithms combining the theory of point processes and adaptive estimation to study neural plasticity during learning in both the rodent and monkey medial temporal lobe regions. The experimental investigations will systematically study the dynamics of neural activity within the hippocampus and adjacent medial temporal lobe structures (entorhinal, perirhinal and parahippocampal cortices) in rats, genetically altered mice, and primates. These experimental studies will provide the basis for a focused investigation that develops neural signal processing methods appropriate for dynamic analysis of multiple simultaneously recorded neural spike trains. The algorithms we develop will be used to analyze the data collected in the experimental studies proposed in this investigation. The close collaboration between the experimentalists and the quantitative scientists will ensure that the methods designed are appropriate for the data collected. The objectives of this partnership are to provide a careful quantitative description of neural plasticity and how it relates to learning, memory formation and behavior, and to develop broadly applicable signal processing tools for analyzing the dynamic behavior of neural ensembles.

STATUS OF RESEARCH AND PARTNERSHIP:
The first year of the project has been very productive with significant progress having been made on all of the specific aims.
Specific Aim 1: Dynamic Analysis of Information Encoding within the Hippocampus (Matthews A. Wilson, MIT)
We have begun our experiments involving the simultaneous recording of CA1, CA3, and DG neurons during exposure to familiar and novel environments, with data collected from one animal and a second animal in progress (Specific Aim 1A-B). Data has also been collected and initial analysis completed on another existing mutant line (PSD-95 KO) that displays learning deficits along with enhanced LTP of hippocampal synapses. Our initial analysis of spatial receptive field properties and dynamics in these animals and have established a novel phenotype that relates to receptive field shape characteristics and dynamics that were proposed to be studied in specific Aims 1A-C. This initial work will soon be prepared for publication. Further data collection and analysis of these animals are underway. Initial behavioral studies and behavioral hippocampal recordings are being conducted on a new line of DG-specific NMDAR-KO animals with preliminary results expected by the fall (Specific Aim 1C). Preparations are being made to begin collecting similar data from mice with CA3-restricted genetic deletion of NMDA

receptors. These experiments should begin in the fall with additional personnel hired to carry out this work (Specific Aim 1C).

Specific Aim 2: Dynamic Analysis of Information Encoding Within the Hippocampus and Adjacent Regions of the Medial Temporal Lobe (Wendy Suzuki, NYU).

We have made important progress in understanding the dynamic analysis of information encoding both in the hippocampus as well as the adjacent cortical areas. We recently published a report detailing the patterns of activity in the hippocampus during the formation of new associative memories as animals performed a location-scene association task (Wirth et al., 2003a). We report that hippocampal neurons signaled new learning with dramatic changes in their stimulus-selective response properties. We call these cells "changing" cells. Because cells change before, during and after learning occurred, this suggests that the hippocampal cells participate in the early formation of new associative memories. We have not only extended these findings to another associative learning task in monkeys (object-place association task; Wirth et al., 2003b), but we also have evidence that similar kinds of dynamic changes occur in humans performing the location-scene association task (Law et al., 2003). We are also pursuing more detailed analysis of the fundamental rules governing associative learning in monkeys (Chiu et al., 2003). We have also made important progress in analyzing the associative learning related signals in the adjacent perirhinal cortex (Yanike et al., 2003). We report that changing cells are also observed in the perirhinal cortex during learning, but the changing cells in the hippocampus tend to change after learning has occurred, whereas many hippocampal neurons change before or at the same trial as learning. These findings suggest that while some hippocampal cells may be involved in the early formation of new associations, other cells in the hippocampus and perirhinal cortex may be recruited after learning to help strengthen the newly formed memory.

Specific Aim 3: Dynamic Signal Processing Methods for the Analysis of Neural Plasticity (Brown, MGH/HMS; Solo, UNSW).

We have made progress on the development of the algorithms described in this specific aim. We described in Smith and Brown (2003) a new paradigm for estimating the state space models from point process observations. This theoretical paradigm served as the basis for the dynamic estimation algorithm we used in Wirth et al. (2003) to estimate the behavior learning curve and relate it to changes in neural spiking activity of neurons in the monkey hippocampus. We just completed and submitted a detailed development of the learning curve estimation algorithm (Smith et al., 2003). The software to implement the algorithm is available on our website at http://neurostat.mgh.harvard.edu/Learning. We also completed and submitted for publication manuscripts on a general neural spike train decoding algorithm (Barbieri et al., 2003) and a general paradigm for adaptive estimation from point process measurements (Eden et al., 2003). The decoding algorithm will be applied to data from Specific Aim 1, whereas the adaptive estimation algorithm will be applied to data from both Specific Aims 1 and 2. We have also applied the dynamic estimation algorithms to the analysis of another neural signal namely human heart beats (Eden, Barbieri and Brown, 2003).

Significance

Specific Aim 1: We have demonstrated the ability to identify novel receptive field dynamics and characteristics that can be related to behavioral performance through the application of genetic manipulations. We have also demonstrated the ability to successfully monitor the simultaneous dynamics of receptive field characteristics in 3 hippocampal subregions (DG,CA3,CA1).

Specific Aim 2: The findings related to Specific Aim 2 have provided some the first and strongest direct evidence of learning-related plasticity in the medial temporal lobe in behaving animals.

Specific Aim 3: The accomplishments this year demonstrate theoretically and in the analysis of actual experiments that the dynamic estimation methods for point processes can be developed and successfully applied to characterize behavioral and neural dynamics.

ISSUES:

We have not encountered any issues that have impeded the progress of our research.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Child Health and Human Development (NICHD)

PROJECT TITLE: FES and Biomechanics: Treating Movement Disorders

ABSTRACT:

This multi-investigator project combines resources from four professors of Mechanical Engineering and three professors of Physical Therapy through our newly organized Center for Biomedical Engineering Research at the University of Delaware. The five-year goal of this project is to assist patients with CNS dysfunction to produce improved walking patterns through a combination of functional electrical stimulation (FES), robotic-assistive training and biomechanical modeling. In the first phase of this project, which is described in this proposal, the focus will be on individuals with stroke exhibiting hemiparetic leg impairment. The technique should be generalizable to a variety of neurological impairments. The movements for these individuals will be improved or "optimized" in four ways: Nonrisk--Maximize postural stability, Injury--Minimize musculoskeletal injury (e.g., arthritis) during movement, Cosmesis--Develop a more natural looking gait, and Energy--Minimize metabolic energy consumption during movement. The NICE optimization protocol will be realized through musculoskeletal modeling, robotic assistance, functional electrical stimulation, and neuromuscular training. The specific task we will study will be partial body weight suspension gait on a treadmill. The organization of this project has been divided into 3 distinct aims, which may be summarized as follows. Aim 1: Identify impairments in the locomotor patterns of the lower extremity in patients with hemiparetic stroke and create a paradigm to optimize the movement patterns ("NICE" optimization). This will be accomplished through biomechanical modeling using gait analysis and electromyographic data. Aim 2: Develop the methods and equipment "NICE" rehabilitation system) necessary to implements the "NICE" optimization of locomotion in patients with stroke. We will achieve this through the use of a robotic device and an electrical stimulation system. Aim 3: Test the feasibility of the use of the "NICE" rehabilitation system in patients with hemiparetic stroke and make adjustments to the system based on the patient trials. Our ten-year goal is to produce a portable (wearable) FES system to assist patients with CNS dysfunction in the production of coordinated movements.

STATUS OF RESEARCH AND PARTNERSHIP:

Biomechanical modeling: We have created an improved method to estimate muscle activation from EMG signals. This method involves a mathematical model with a single adjustable parameter that fit data reported in the literature better than previously methods. We have observed that by skewing the force-length curve as a function of muscle activation, substantially better fits to the data are observed. We implemented this and compared many different methods to optimize performance and found that as few as seven adjustable parameters could be used to adequately model a complex joint.

FES: A revised FES model is presently being tested on a group of healthy subjects that include the ability to predict changes in muscle force in response to changes in stimulation intensity. We have also begun to work on the hardware to be used to stimulate the patients' muscles and are presently modifying the stimulator to allow our computer to control the precise timing and duration of the stimulation pulses to be delivered to the patients.

Robotics: We are developing gravity-balanced leg orthoses for the human leg that can fully or partially balance the leg over its range of motion. The first prototype was targeted at two DOF motion of the leg in the sagittal plane, i.e., single DOF flexion and extension at the hip and knee. We also developed the theory and design of a gait corrective orthosis using cam-follower theory using normal motion of the hip and knee during a walking cycle.

ISSUES:
There are no issues that have arisen in regard to our partnership. In regards to the science, we have decided to reconsider the use of a treadmill in this study of human gait. We believe that a more realistic gait may be obtained during ground-based walking and have devised a means to accomplish that goal using a robot that would move with the person. This is a minor change to the goals of the project, but represents a major change in the robot design. However, this is coming along very well.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB)

PROJECT TITLE: Combined Digital X-Ray and Ultrasound Breast Imaging

ABSTRACT:
Mammography and ultrasound have been used extensively in breast imaging over the last few decades, but in separate examinations, with the breast in substantially different positions. Ultrasound acquisitions have predominantly been through manual manipulation of ultrasound probes in direct contact with the breast. Due to the different geometric configurations of the breast, normal fibrocystic changes occurring over a period of time, possibility of structures being occult on one modality and visible on the other, and operator dependency of US image quality, accurate matching of regions of interest on the two sets of images has been very challenging. Indeed this culminates in sub-optimal joint evaluation of the two modalities. Earlier attempts by several investigators to provide a means of acquiring the images in a spatially correlatable manner met with limited success, in part due to inferior imaging technologies, and in part due to inadequate or impractical means of interfacing the two modalities by associated choices of system designs.

The first goal of this project is to leverage recent and steadily improving technological advances in mammography and ultrasound by designing and evaluating a common compression paddle platform to interface the two imaging systems. These advances include digital mammography, broad-band, high frequency, multi-row linear array ultrasound transducers with capabilities of providing multiple focal zones at reasonable frame rates for real time imaging. The platform will enable co-registered acquisition of breast images with the two modalities in a single examination, in the same position, and in a semi-automated manner to mitigate the dependency of image quality on operator skills. Major challenges in the design of the platform include the requirement of obtaining sufficient breast coverage with both modalities while maintaining strict clinical image quality constraints in an automated, reproducible manner. Initially, the diagnostic results should be equivalent to those obtainable with the two modalities independently. The platform will be evaluated by seven MQSA certified mammographers conducting a comparison between diagnostic mammograms and manual, expert-performed full breast ultrasound as the baseline. The second goal of this project is to leverage this platform to develop and compare advanced applications based on digital mammography and ultrasound, such as tomosynthesis, compound imaging, elasticity imaging, vascularity imaging and multi-modality CAD. While some of these modes have been researched independently by several investigators,

no cohesive studies demonstrating relative clinical benefits of these modes have been performed. With the common compression platform, these modes will be evaluated and directly compared on the same patient, in the same geometry and in the same examination, for a total of 120 patients, some scanned twice and 60 others imaged in development studies. The overall goal is to assess combinations of modes that may lead to improved accuracy in breast imaging.

STATUS OF RESEARCH AND PARTNERSHIP:

Digital X-ray/Ultrasound Breast Imaging System: The GE team is directed toward modifying the original prototype paddle system described above for short studies at the University of Michigan in July 2003. Two paddle styles include a rigid plastic plate and a tight membrane. Conformal transducer tracking, beamforming corrections and external triggering have been implemented on the new Logiq 9 ultrasound scanner. Dedicated, multimodality visualization software is available. U of M team basic system efforts include redevelopment of an earlier GE prototype standalone scanning system; testing of image quality; consulting on system design; preparation for preliminary and main trials.

Advanced Imaging Modes: Nonlinear Elasticity Imaging -- We are investigating 3-D nonlinear elasticity imaging of the breast for surface deformations by the compression paddle to enhance the contrast of traditional elasticity images. Full finite element simulations of nonlinear elastic 3D material geometry has been developed to provide synthetically generated 3D RF data volumes for developing, testing, and optimizing speckle tracking and image registration algorithms. Vascularity Analysis -- The goal is to assess Doppler vascular imaging as a function of mammographic compression, possibly to the point of isolating shunting and other vascular anomalies. Progress is good toward reasonably high speed 3D Doppler imaging and fine compression control and readout. Compound/Multiview Imaging and X-ray Tomosynthesis -- System design has been performed to allow further development of these techniques and conduct of clinical studies.

Advanced Image Processing: Preparations are being made for image based registration of volumetric and projection image sets and CAD using combined images.

ISSUES:

There are no issues of particular concern. Both partners are aggressively working towards meeting the goals stated in the program, despite a late start. We have active discussion forums between the partners on a weekly and monthly basis, respectively, and on a needs basis as well. The program commenced with a special session in which radiologists, physicists, ultrasound/mammo technologists and engineers from the University of Michigan and GE Global Research participated to flow down the top level clinical requirements of the program to system and sub-

system requirements. This greatly helped prioritize tasks according to those that would have the greatest impact on the requirements for the BRP program, and will provide the right direction for execution. Five abstracts have been submitted for past or upcoming conferences based on joint work since initiation of the grant.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB)

PROJECT TITLE: Optical Biopsy Using MEMS Technology

ABSTRACT:
The broad, long term objective of the proposed research is to develop a noninvasive system for optical biopsy using microelectromechanical system (MEMS) technology. We propose to combine the advances in biomedical imaging and MEMS technology to develop a high speed, endoscopic functional optical coherence tomography (OCT) with a miniaturized probe for early diagnosis of lesions and tumors in gastrointestinal (GI), respiratory, and urogenital tracts.

The specific aims of this work are to: (1) design and develop a high speed, fiber optic based high resolution functional OCT system for endoscopic imaging of in vivo tissue structure and blood flow dynamics in GI tracts, and investigate and develop hardware systems and imaging processing algorithms for speckle noise minimization and imaging enhancement (Chen); (2) design and develop scanning probes with silicon MEMS technology (Tian); (3) design and develop scanning probes with polymer MEMS technology (Li and Bachman); (4) integrate MEMS probe with OCT system and perform in vitro and in vivo testing (Chen, Tien, Li, Bachman, Chang); and (5) investigate the applications of MEMS based endoscopic OCT for early diagnosis of lesions and tumors in GI tracts (Chang and Chen). This is a collaborative project that involves PI and Co-PIs with expertise in biomedical optics, silicon and polymer MEMS technology, and endoscopic imaging. The scanning probes developed using MEMS technology have the advantage that they are compact, robust, low cost, low power requirement, and high speed. In addition, lateral resolution of the current endoscopic OCT that uses axial scanning followed by lateral scanning is limited by the focal depth of the probe beam. The high scanning rate of the probe made with MEMS technology offers the potential to increase lateral resolution by performing lateral scanning first in order to maintain the beam waist at the zero optical path length. Furthermore, a scanning probe fabricated with MEMS technology has the potential to provide three-dimensional imaging of tissue structure and physiology with high imaging speed. Finally, the scanning probe technology developed in this proposal can also be used for endoscopic confocal and two-photon imaging.

STATUS OF RESEARCH AND PARTNERSHIP:
In the first year of this project, significant progress has been made in the advancement of functional OCT technology, silicon and non-silicon MEMS probe devices.

A major effort has been the development of high resolution OCT imaging using broadband light sources that uses continuum generation from photonic crystal. High resolution OCT with a longitudinal resolution of 1.3 microns in tissue at a center wavelength of 1.1 microns was demonstrated. Sub-cellular imaging using high resolution OCT has now been demonstrated.

Significant advances have been made in silicon, polymer and metal based MEMS devices for the endoscope probe. Silicon mirrors were using a novel microfabrication process using thinned single crystal silicon with thick stiffening backbone structures. Actuation is provided by stacked vertical comb-drives and the devices are optimized for operation at resonance. 500 micron mirrors were fabricated. A driving voltage of 50 V achieved a scan angle of 20 degrees for designs with frequencies between 1 kHz and 10 kHz.

Nickel mirrors 700 microns wide were batch fabricated and actuated using both magnetic and hydraulic forces. A magnetic resonant scanning mirror system was scanned to over 35 degrees at a resonant frequency of 345 Hz. A polymer based microfluidic system was demonstrated to repeatedly actuate a mirror to 25 degrees at low frequencies (less than 50 Hz) with high force capability.

The partnership is functioning very well. Investigators regularly visit each other's laboratories, hold biweekly joint group meetings, and their students utilize both laboratories for their research.

ISSUES:
We are working with UCI IRB for the approval of the human subjects protocol. One of the concerns the committee raised is that they would like to see some animal testing results, which we proposed to do in the second and third year. In our proposal, the human subjects will not be involved until the fourth year. Therefore, we should have sufficient time to get results from the animal studies, which have been approved by IACUC.

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung and Blood Institute (NHLBI)

PROJECT TITLE: Prevention of Hemodialysis Vascular Access Stenosis

ABSTRACT:
Neointimal hyperplasia is a frequent cause of stenosis in blood vessels and is commonly observed in post-angioplasty coronary arteries and hemodialysis vascular accesses. In native arteriovenous (AV) fistulae and polytetrafluoroethylene (PTFE) grafts for hemodialysis, the stenosis is usually focal and occurs at the AV or graft-venous anastomosis. Effective strategies for the prevention of stenosis are lacking. We hypothesize that local delivery of anti-proliferative drugs and anti-growth factor antibodies using a novel drug delivery system, ReGel, will inhibit neointimal hyperplasia associated with native AV fistulae and PTFE grafts. ReGel is an injectable, thermosensitive copolymer designed for local, sustained-delivery of drugs.

This is a multidisciplinary approach to address the following specific aims: (1) To examine the efficacy of two anti-proliferative drugs (dipyridamole or paclitaxel) and anti-platelet derived growth factor (PDGF) antibodies alone or in combinations in the inhibition of growth of human or canine vascular smooth muscle cells. These in vitro studies will set the stage for animal studies in this proposal and potential clinical trials in the future. (2) To study the release kinetics of the anti-proliferative drugs and anti-PDGF antibodies from ReGel in vitro and their transport kinetics across explanted native AV fistulae and PTFE grafts. The transport characteristics of the drugs and antibodies in ReGel applied to the perivascular area of the native AV fistula and PTFE graft around the venous anastomosis will then be evaluated in whole dog experiments. Comparisons of mathematical model predictions with results from these experiments will help optimize the therapeutic dose of drugs and antibodies and conditions for delivery by ReGel in vivo. (3) To examine the efficacy of the anti-proliferative drugs and anti-PDGF antibodies delivered by ReGel in inhibiting neointimal hyperplasia in dog models of native AV fistula and PTFE graft.

Successful development of this technique will provide a novel approach of local drug delivery to prevent neointimal hyperplasia and stenosis in blood vessels. Furthermore, the results will provide the basis for local delivery of drugs and proteins of interest to a variety of tissues.

STATUS OF RESEARCH AND PARTNERSHIP:
Dr. R Rathi, who had been participating from the beginning, has assumed Dr. Zentner's role at MacroMed, Inc. on our project. The consortium agreement otherwise remains unchanged. From the Utah Artificial Heart Institute (UAHI), Dr. S. Mohammad is now a part-time employee at the VA Medical Center and Dr. G. Burns is a full time University of Utah employee as of June, 2003. Since these essential personnel are now closely affiliated to the University, we have dissolved the consortium agreement with the UAHI, but retained Dr. D. Olsen, Director of the UAHI, as a

consultant on the project. The involvement of Dr. Mohammad remains unchanged and Dr. Burn has now assumed a greater role in the animal experiments and histology in this project.

For Specific Aim 1, we have been concentrating on the mechanism of action of dipyridamole and imatinib on vascular smooth muscles. For Specific Aim 2, we have set up an in vitro model to study the kinetics of dipyridamole transport across the PTFE graft wall. In addition, we have formulated more concrete plans to study the in vivo kinetics of drug through the interstitium and across the PTFE graft using autoradiographic techniques. For Specific Aim 3, we have injected 0.26 mg of paclitaxel combined with ReGel to the venous-graft anastomosis at 2 weeks after placement of femoral grafts in 8 dogs. Preliminary results are very promising, in both safety and efficacy. More detailed morphometric analyses of anastomoses, including 3-dimensional reconstruction, are being established.

ISSUES:

There are no major issues encountered. Perhaps the most significant issue relates to the procurement of PTFE grafts. The previous vendor had been donating the grafts to us for several years. Therefore, the costs of the grafts, which are quite high, have not been budgeted in the grant application. A few months ago, this vendor has ceased providing us with their grafts, because of issues related to commercial interests. So far, we have been able to obtain PTFE grafts from another vendor, who has actually assisted us in solving the problem of kinking by providing us with grafts that have external spiral enforcement. There are no problems with the consortium arrangement. The multi-disciplinary approach, involving pharmaceutics (Dr. Kerns, Dr. Kim) polymer chemists (Dr. Rathi and colleagues at MacroMed), bioengineer (Dr. Leypoldt), animal experiment experts (Dr. Olsen and Dr. Mohammad), pathologists (Dr. Burns and two University core facilities) has functioned very well in this project.

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute (NHLBI)

PROJECT TITLE: Molecular Basis of Endothelial Remodeling by Flow

ABSTRACT:
Hemodynamic forces regulate the structure and function of the blood vessel wall. Vascular endothelial cells (ECs) are exposed to shear stress, the tangential component of the hemodynamic forces acting on the vessel wall. ECs in the straight part of the arterial tree are subjected to laminar flow with high shear stress, whereas cells in the bends and bifurcations are under disturbed flow patterns with low shear stress. Our hypothesis is that the preferential localization of atherosclerosis in the branch points of the arterial tree and the sparing of the straight parts can be related to the different molecular responses to these flow patterns. The laminar flow in the straight part of the vessels is anti-atherogenic by arresting the EC cell cycle. In contrast, disturbed flow at branch points is pro-atherogenic by increasing EC proliferation. Laminar flow also enhances the repair of the dysfuntional endothelium by augmenting EC migration, whereas disturbed flow retards the repair by inhibiting cell migration. We will test our hypothesis that laminar flow and disturbed flow activate different molecular signaling pathways to result in the expression of unique sets of genes, thus leading to the opposite functional consequences of anti-atherosclerosis and pro-atherosclerosis, respectively. The research design has three Specific Aims. In Specific Aim 1, we will establish the molecular basis of the arrest of EC cell cycle by laminar flow and the enhancement of the EC proliferation by disturbed flow. In Specific Aim 2, we will elucidate the molecular mechanisms by which EC migration is modulated by laminar and disturbed flows. In Specific Aim 3, we will identify the genes regulated by laminar flow and disturbed flow by using DNA microarray chip technology, with the aim of guiding in-depth studies on the flow-responsive genes that modulate EC growth arrest, proliferation, and migration. The proposed research involves partnership among scientists with expertise in vascular biology, physiology, biomechanics, bioengineering, bioinformatics, cell biology, and molecular biology. This interdisciplinary research program will allow us to elucidate the molecular basis of flow-induced modulation of EC turnover and migration, which are two important functions for vascular remodeling. The results will serve to generate new knowledge on mechano-transduction and vascular biology, provide new understanding of the molecular and biomechanical bases of pathogenesis of vascular disorders such as atherosclerosis, and help to develop new therapeutic strategies.

STATUS OF RESEARCH AND PARTNERSHIP:
Excellent advances have been made under all three Specific Aims.

1. We have studied the molecular basis of the arrest of EC cell cycle by laminar flow and the enhancement of the EC proliferation by disturbed flow. We found that sustained laminar shear stress causes a sustained p53 activation, which induces the up-regulation of GADD45 and p21. The resulting inhibition of cyclin dependent kinase and Rb hypophosphorylation lead to the EC cell cycle arrest at G0/G1.

2. Drs. Schwartz's and Chien's labs have collaborated in studies on the molecular mechanisms of flow-modulation of EC migration, including the roles of the small GTPases Rho, Rac and cdc42 and their relations to cytoskeletal, junctional and matrix proteins. Molecular manipulation has been combined with micromechanical techniques to elucidate the interplay between molecular alterations with mechanical forces in governing cell migration. We have compared the migration of ECs during the wound healing in response to laminar and disturbed flows.

3. Using the DNA microarray technology, we have found that 24-hr of laminar flow down-regulates a number of genes related to inflammation and EC proliferation and up-regulates several genes involved in EC survival, angiogenesis, and vascular remodeling. Collaborative microarray studies between Drs. Guan's and Chien's labs on effects of molecular manipulation of focal adhesion kinase (FAK) on gene expression profile have shown that FAK causes an up-regulation of the transcription factor KLF8.

Twenty-two papers have been published in peer-reviewed journals, including seven joint publications between the participating institutions under the BRP.

ISSUES:
Dr. Martin Schwartz moved from The Scripps Research Institute to University of Virginia in 2002. The collaboration under the BRP has continued very well.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB)

PROJECT TITLE: Uropathogen detection using DNA biosensors

ABSTRACT:
Urinary tract infection is the most common urological disease in the United States and is a major cause of patient morbidity and health-care expenditure. This Bioengineering Research Partnership proposal involves development and testing of a system for the genotypic detection and species-specific identification of uropathogens within a time frame (5-10 minutes from sample collection to readout) that would enable point-of-care diagnosis and treatment. The focus of this proposal is to develop a self-contained microbial pathogen detection device and to examine its performance using clinical urine samples. Research at UCLA has provided two key technological advances that make development of a uropathogen sensor feasible. The first is microfluidics for sample processing. The second is an electrochemical microsensor that allows ultrasensitive detection of specific DNA-RNA or DNA-DNA hybridization events, without the need for target amplification. Specific Aim 1 describes how microfluidics studies will be applied to development of a filter for uropathogen concentration, micromixing for processing of uropathogen nucleic acids, and washing of the sensor surface. Specific Aim 2 involves fabrication of the microsensor array, development of a streptavidin self-assembled monolayer, and testing of oligonucleotide probes for electrochemical detection of uropathogen rRNA and mRNA on the microsensor surface. Specific Aim 3 will involve integration of the microfluidics and sensor components and testing of its analytic validity on simulated and actual urine specimens. Specific Aim 4 will involve fabrication of sufficient numbers of the device to test the association between urosensor results and clinical correlates of urinary tract infection.

STATUS OF RESEARCH AND PARTNERSHIP:
Organization of the Project. Several organizational procedures were put in place to facilitate communication between members of the research group. All personnel involved in the project are required to attend a monthly research meeting, submit both verbal and written reports, and are invited to submit items to the meeting agenda and to review minutes from the previous meeting. Each of the research groups submits both a verbal and written report describing their goals for previous month, methods, results, problems, and plans for the coming month. We have created a "Uropathogen Detection Using DNA Biosensors" website:

http://www.urology.medsch.ucla.edu/uropathogendnabiosensor-home.html

Microfluidics. Dr. Chih-Ming Ho's group at the UCLA School of Engineering has had two major goals during the first year of the project. The first microfluidics goal was to develop a "Biofilter" to capture and concentrate bacteria from a liquid such as urine. Two Biofilter approaches have been developed using either "dielectrophoretic" (DEP) or AC electro-osmotic forces. Both of these forces use an interlocking array of electrodes that create a nonuniform electric field that induces a dipole moment on any uncharged dielectric and/or conductive particle (such as a bacterium) in the fluid. Both forces are active at a range of 100s of microns, consistent with the microfluidics requirements of a urine Biofilter. Once the dipole moment is induced, the particle is drawn by the electric field and is captured on the electrode surface. This would allow small numbers of bacteria to be captured from a large volume of liquid, residual urine washed away, and then the bacteria would be released in a small volume of the lysis solution. The effectiveness of DEP and AC electro-osmotic forces depends on the conductivity of the fluid and size of the particle to be captured. Since DEP requires relatively low conductivity, AC electro-osmotic force would be the most appropriate choice for this application. The second microfluidics goal was to develop the micromixing technology for the uropathogen detection system. A prototype automated sample preparation system has been designed and fabricated using micromachining of an acrylic chip measuring 2.4" x 3.7". The chip contains ports for a peristaltic pump and valves controlling delivery of bacterial DNA, oligonucleotide probes, wash solution, and enzyme substrate to the sensor surface. Micromixing in a serpentine channel incorporated into the chip has been documented by video microscopy.

Probe Design and Testing. Our first goal was to create the UCLA Uropathogen Specimen Bank containing a representative collection of 400 uropathogens isolated from inpatient and outpatient urine specimens. Consecutive isolates of uropathogenic bacteria were obtained with sufficient numbers of E. coli, Proteus, Klebsiella, Pseudomonas, Enterobacter, and Enterococcus species for statistical analysis of sequence diversity. The rrs gene encoding the 16s ribosomal subunit of over 70 uropathogens has been amplified using universal bacterial primers. Alignment of 16s sequences of 27 E. coli uropathogens revealed > 98.7% sequence identity and there was no sequence variation among the E. coli sequences in the hypervariable region that is the most useful for species-specific capture-detector probes. Using this information, we have designed and tested probes that show species-specific hybridization at room temperature, an achievement which will avoid the need to place heating or cooling elements in the urosensor device.

Electrochemical Sensor. Development of the electrochemical sensor is under the direction of Dr. Vincent Gau at GeneFluidics, Inc. To ensure the quality of the sensor chip and improve the sensitivity, GeneFluidics has established a standard operation procedure for quality control of the nanometer scale chemical structure at the surface of electrochemical sensor. This well-defined surface served as an interface between the transducer and the biological environment and is the key element for ultra-sensitivity and reproducibility. GeneFluidics has developed an analytical method to monitor the total impedance and capacitive information of each sensor with a fast low-cost approach and it has been used to screen for sensor defects. This has greatly improved the sensitivity of the sensor by eliminating transducer factors. Currently, the sensitivity of the sensor is one femto molar. Since GeneFluidics' sensor fabrication is very different from traditional manufacturing, any contamination would affect the sensitivity for very low concentration detection. In the past year, we have identified several types of metal or organic contamination from the manufacturing production line by using this analytical QC procedure and actions have been taken to eliminate the contamination.

ISSUES:

The BRP granting mechanism has greatly facilitated this multidisciplinary effort, which cuts across traditional academic and institutional structures.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)

PROJECT TITLE: Engineering Aspects of Liver Support Systems

ABSTRACT:
In spite of many advances in liver transplant surgery, an increasing number of patients with terminal liver disease are dying while awaiting a transplant. Consequently, further advances in the storage of donor livers, as well as alternative replacement options are needed. A very promising area of research and development is in the development of engineered solutions to the problems of liver support. However, efforts undertaken within a single discipline are hampered by the complexity of both the engineering and biological aspects of such projects. This proposal constitutes a partnership between bioengineers and biologists with the goal of combining their expertise to devise improved methods of liver support via bioartificial livers and improved preservation of donor livers via machine perfusion preservation (MPP). The partnership encompasses three inter-related projects. The first project focuses on deliver of oxygen and other nutrients to the cell in in vitro systems such as the bioartificial liver. The approach involves the modification of the support matrix to facilitate enhanced mass transport. The second project addresses the hypothesis that improved bioartificial liver function can be attained by providing a more physiological combination of cell types in the support device. Specifically, we will investigate the relationship between Kupffer cells and hepatocytes in maintaining prolonged hepatic-specific function in culture. The final project focuses on development of methods for optimization of microvascular perfusion and oxygen delivery in pump perfused livers. This project uses a combination of intravital microscopy and mathematical modeling. In all of the projects, engineering and biological approaches are combined to address focused, clinically relevant problems. Moreover, the unique environment that supports the partnership will maximize the potential for success in this interdisciplinary approach.

STATUS OF RESEARCH AND PARTNERSHIP:
In this second year of funding of the partnership the function of the partnership has begun to show excellent productivity. We had 8 publications or reviewed congress presentations and are making excellent progress in all components of the partnership. Particular progress has been made in projects 1 and 3. Project 1 progress during Year 2 has chiefly been made on Specific Aims #1 and #4. This effort consisted chiefly of engineering two in vitro systems for testing how O2 transport within the enhanced collagen extracellular matrix (ECM) can be manipulated to expand the dimensions of the BAL's cellular space; culturing adult rat hepatocytes within the BAL prototypes of our collaborators at UNC-Chapel Hill; and measuring the viability and functional performance of the hepatocytes within the BAL. Furthermore, we are in the early

stages of quantifying the relationship between the level of O2 supplied and the efficiency in which it is used by the cells in order to optimize the cell viability and function in this system (Specific Aim 2).
In project 3 we have made significant progress in the areas of determining the interaction between cold temperature and shear forces on function of sinusoidal endothelial cells in culture and on the effects of prolonged machine perfusion on the permeability and morphology of sinusoidal endothelial cells in the intact isolated perfused livers. We have also extended our studies to examine the efficacy of machine perfusion on resuscitation of marginal donor livers. We have shown that machine perfusion improves survival of rats with transplanted livers from nonheartbeating donors for 0/7 for simple cold storage to 5/6 for machine perfusion. We are now pursuing mechanistic studies to explain that observation.

In project 2 work has focused on fabrication of templates for patterning of different cell types for coculture experiments and the development of methods for characterization of hepatocyte specific function. Briefly, the technique uses photoresist on borosilicate glass to create the "masters" for preparing polydimethylsiloxane (PDMS) membrane templates. The templates are then placed in 35 mm tissue culture dishes, the extracellular matrix is added, and the plates are sterilized in preparation for cell seeding. The final characterization of the micropatterned surfaces is currently being completed using profilometry and SEM. Toward SA #2, we have successfully prepared various micropatterns to ultimately determine the arrangement of hepatocytes to kupffer cells that yields the best hepatocyte function. Thus far we have successfully cultured hepatocytes and kupffer cells on the micropatterned surfaces, and we are currently evaluating morphological changes in time using videomicroscopy. Simultaneously, we have begun work on SA #2 and are currently evaluating albumin and urea secretion as well as PEPCK levels to quantify the effects of the micropatterned co-cultures on hepatocyte performance.

ISSUES:
The partnership has functioned remarkably well largely because of at least biweekly meetings of the all partners as well as students and technicians working on the projects. It continues to be a challenge to maintain an effective interface between the engineering and biological aspects of the projects, but the frequent interdisciplinary meetings have substantially improved the understanding of the biology on the part of the engineers and the engineering on the part of the biologists. The input of our clinical collaborator has also been extremely valuable for the continued focus of the projects on ultimate clinical application. Work supported by the partnership has already resulted in additional applications (one RO1 and one SBIR) by the partners to support projects based upon the findings of the BRP grant and the collaborative relationships established in the partnership. The success of the partnership has also served as the focus for the planned establishment of an Institute for Biomedical Engineering Systems on the UNC Charlotte campus.

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung and Blood Institute (NHLBI)

PROJECT TITLE: Molecular and Cellular Mechanisms in Cardiovascular Tissue Engineering

ABSTRACT:
This is a multi-investigator proposal from a single campus that addresses fundamental bioengineering mechanisms in cardiovascular cells and their preclinical application in vivo and ex vivo. We investigate the cell and molecular mechanisms by which the local physical and chemical environment regulates cardiovascular cell and tissue physiology and integrate this with testing and implementation of engineering principles in tissues and experimental animals. This is particularly important in the cardiovascular system where the biomechanical, structural, and chemical environments are spatially complex. The program addresses both hypothesis-driven and design-driven experimental approaches in varying proportion. The BRP investigators, a mix of biomedically-trained and engineering-trained faculty, share a strong commitment to interdisciplinary research and represent a community of multidisciplinary scholars. Most of the program is physically located at Penn's Institute for Medicine and Engineering (IME), which was established to connect Medical School and Engineering School scientists working at the interface between biomedicine and the engineering, physical, and computational sciences.

STATUS OF RESEARCH AND PARTNERSHIP:
The partnership crystallized from associations that had been assembled shortly before funding and we were therefore able to become productive quickly. By the end of the first year, 14 full length papers supported by the grant were published or in press. In the latter part of this second year (June 2003) an additional 15 papers are published or in press.

Each investigator addresses one major specific aim in which substantial bioengineering expertise is essential to test hypothesis-driven biomedical research that in turn influences the success of pre-clinical testing. The approach taken in this proposal is to seek new knowledge of mechanotransduction mechanisms at a cell and molecular level that will (i) promote the conception, development, or improvement of therapies, and (ii) permit an understanding of why certain existing appproaches work. For example, spatial imaging of cytoskeletal networks, fluid dynamics, force measurements, mass transport calculations, and complex biophysical measurements are directed towards the development and optimization of therapeutic intervention.

Highlights of this year include AFM studies of forced extension and unfolding of proteins to reveal the effects of chemical modifications, single living cell biomechanical measurements both as spatial strain mapping inside the cell and extracellular traction forces, matrix-integrin

regulation of phosphatases, mechanically-responsive ion channel identification, heart valve gene expression and biochemistry, endovascular microcoil gene delivery, and the mechanical regulation of vein graft remodeling.

ISSUES:
The partnership benefits from a contiguous campus and seamless daily interactions of the investigators. There is a monthly BRP presentation-discussion meeting as well as presentation of the work within the regular infrastructure of two Institute and departmental seminar series and Institute Chalk Talks.

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GRANTING NIH INSTITUTE/CENTER: National Center for Medical Rehabilitation Research (NCMRR)

PROJECT TITLE: Harnessing Motoneuron Activity: From Lab to Clinic

ABSTRACT:
We propose to develop an automatic system for decomposing the electromyographic (EMG) signal into the constituent action potentials corresponding to the firing of individual motor units activated by motoneurons. The system will be an outgrowth of our existing rudimentary system, which over the past 20 years has enabled us to perform various novel investigations that have provided a variety of new insights into motor control. However, the current system suffers from many limitations, which curtail its usefulness as a research tool, and has never been useful as a Clinical Tool. The new system will have a dramatically enhanced performance: 1) decomposition time for typical contractions will be decreased from dozens of hours to a few minutes, 2) the automatic decomposition accuracy will be increased from 60 % to 95% - with provisions for assisted editing to reach 100% accuracy, 3) it will be able to decompose signals from dynamic as well as static contractions (which is a current limitation), 4) it will weigh less than 10 kg, and will have a notebook computer configuration, and 5) most importantly the decomposition algorithms will be completely rewritten using a newly developed knowledge-based Artificial Intelligence language blackboard platform developed by us. This platform has been used successfully to decompose polyphonic signals and radar spread spectrum signals having a complexity comparable to that of the EMG signal. The proposal is composed of 5 projects. The first and dominant project will be Design Driven. It describes the design and development of the new system, which has at its heart, a Knowledge-Based algorithms for decomposing the signals. The other four projects will be hypotheses-based and will address basic science questions and clinical applications that will reveal the utility of the new system. These projects will also be used to test and improve the evolving design of the new system. Project 2 will address the modifications, which occur in the firing of motor units as a function of Aging. Project 3 will address the phenomenon of motor unit substitution, which will be useful in Ergonomics work environments and in the Rehabilitation of patients with Peripheral Nerve Injury and Spinal Cord Injury. Projects 4 and 5 are two Clinical Studies. Project 4 will explore the use of quantified neuromotor activity for developing prognostic indicators for determining denervation and re-enervation of Paralyzed Laryngeal Muscles. Project 5 will study patients with acute ataxia following a Cerebellar Stroke to explore the manifestation of CNS disorders in the firing characteristics of the motoneurons.

STATUS OF RESEARCH AND PARTNERSHIP:
Project # 1 – Decomposition of the EMG Signal – There are two main components to this project:
Software – The development of the software is on schedule. In FY3 we improved the knowledge-based process that is applied to the results from front-end signal processing and we have made several enhancements to the overall software system in order to provide more robust performance under a greater variety of operating conditions. We have improved the knowledge-based process by incorporating: (1) an utility maximization procedure for rejecting a significant number of false alarms produced by the front-end signal processing, and (2) a multi-path procedure for merging fragments of motor unit trains that are mistakenly classified by the front end as belonging to separate motor units. During the past year, the accuracy of the front end has been improved from an average of 75% to 90%; and the error rate has decreased from an average of 2% to an average of 1%. These improvements have been accomplished while ensuring that the speed of the system is still at least two orders of magnitude faster than the speed of the original system. The increased robustness, under different operating conditions, has been obtained by incorporating two new functionalities into the front end: (1) adaptive segmentation of the input signals, and (2) adaptive adjustment of certain key processing parameters. An "alpha version" of the software system is now being utilized in preliminary clinical investigations. We have also begun building an Editor for the system to allow us to investigate in greater detail regions of undue complexity.

Hardware – The development of the system hardware is on schedule. Two units have been built and one is in 'Beta test" with Dr. Roark at the New York Medical College. The system is configured with a laptop computer linked to a Panel PC. The latter contains custom data acquisition hardware and software; the prior contains the decomposition algorithms. The EMG signal is detected with our existing needle electrodes that are connected to the system via a miniature signal-shaping and signal-quality alert unit. Software programs residing on each computer provide low level-hardware control and a User Interface for seamless data collection, signal processing, and display.

Project # 2 – Aging – As scheduled, this project will end this year. We have executed all the Specific Aims and have achieved the goal of the project. All the data have been analyzed and are currently being interpreted. Preliminary results show differences in the recruitment, the mean firing rate, and firing variability of motor units when comparing young to elderly subjects. The level of cross-correlation between concurrent motor unit activity increased in the elderly suggesting neurological alterations in the control of motor units in the elderly. In light of earlier findings of age-related decreases in the Common Drive in another muscle, our current findings demonstrate that the effects of aging are muscle-specific. In addition to gaining knowledge inherent to the experiment, we were fortunate to gain rare and complex EMG signal patterns that were used to develop and test the Decomposition System.

Project # 3 – Fatigue – The project is on schedule. In FY3 the Vastus Lateralis (VL) muscle was tested in six subjects according to the fatigue-generating protocol. Detailed analysis of motor unit recruitment and firing rate at the beginning, middle, and end of the endurance time revealed that the rules governing motor unit firing behavior remain unchanged with fatigue. This finding, which constituted a Doctoral thesis, together with the known behavior during force modulation forms the basis of a general theory of motor unit control. In a separate, but related series of experiments, an additional seven subjects were tested in the First Dorsal Interosseous (FDI) muscle. Decomposition using the new system is currently under way. We wish to compare the motor unit control properties of the two muscles.

Project #4 – Laryngeal Muscle Control – As scheduled, this project was started in FY3. Dr. Roark has visited the NeuroMuscular research Center on two occasions for discussions on the forthcoming experiments and for training on the Decomposition system. He has implemented preliminary testing procedures in his clinical laboratory at New York Medical College. Four normal subjects underwent simultaneous EMG recordings for the thyroarytenoid and cricothyroid muscles of the larynx and myoelectric responses were elicited by performing vocal and vegetative tasks. This preliminary set of data was processed at the NeuroMuscular Research Center. Results were published in the literature. In sum, many new findings were observed for these muscles, including the presence of Common Drive and ordered recruitment and firing rate control in accordance with force demands. These laryngeal muscles are critical for respiration, swallowing and voice.

Project # 5 – Neurological Studies – As scheduled, this project was started in the middle of FY3. Dr. Manto, who is located in Brussels Belgium has already visited the Center and has received training in the use of the Decomposition system. A detailed review of the recent literature on motor control in cerebellar patients has been carried out. In particular, the scientific papers dealing with the control of limb movements and the recent discoveries in ataxic patients have been selected.

Partnership Collaboration – Dr. Nawab's group and the NeuroMuscular Research Center group have formal two-hour bi-weekly meetings to review progress and to plan future activities. We are in continuous e-mail and telephone contact with our other two partners, Dr. Roark and Dr. Manto. Both have recently been to Boston to criticize the Decomposition system and made several suggestions to improve its functionality. We will be in regular e-mail and telephone contact during FY4. We will exchange personnel during FY4 to facilitate the execution of the experiments and to review progress.

ISSUES:
The collaboration amongst the BRP partners has progressed smoothly and productively.

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GRANTING NIH INSTITUTE/CENTER: National Institute of General Medical Sciences (NIGMS)

PROJECT TITLE: Proteomics to Biomimetic Polymers: Engineering Proteins for Antimicrobials

ABSTRACT:
Our understanding of the structural basis for protein function is rapidly evolving as a result of modern approaches to structural proteinomics. Out intention is to use this understanding as a starting point for the design of biomimetic polymers that are much more stable and inexpensive to produce than natural proteins, but nevertheless mimic their key biological properties. A primary goal of this project will be to design polymer and defined-length oligomers capable of presenting functional groups in arrays similar to those found in natural, biologically active proteins. To illustrate this approach, we will design mimics of a class of membrane-active antimicrobial peptides and proteins. A large class of antimicrobial peptides adopt positively charged amphiphilic alpha-helices, in which changed polar groups and apolar groups line up on opposite faces of the helical cylinder. We recently synthesized a series of mimics of these helices based on betta0amini acids rather than alpha-amino acids. Here, we prose to use these highly simple beta-peptides as frameworks for further elucidating how chains length, helical potential, charge density, and hydrophobicity affect antimicrobial activity. Further, we propose to develop computational methods to aid in the design and analysis of a variety of other antimicrobial polymers that are simpler in structure and hence much less expensive to produce than either alpha- or beta- peptides. The antimicrobial activities of these polymers will be tested in solutions and when attached to solids surfaces. Their structures and mechanisms of action of the polymer and oligomers will be evaluated using a battery of biophysical methods, as well as by using gene chips to examine which genes are turned on by sub-lethal concentrations of the compounds.

STATUS OF RESEARCH AND PARTNERSHIP:
More than 7 classes of polymer backbones have been explored with antibacterial activities. These compounds show excellent activity against several strains of bacteria including E. coli, K. pneumoniae Kp1, S typhimunium S5, P. aeruginosa 10, E. faecium, S. aurues and others. The minimal inhibitory concentrations (MIC) are typically low microgram/ml and, in many cases, hemolysis (IC50) is measured above 400 microgram/ml. This provides substantial selectivity. The ends of the oligomers have been modified to influence the overall hydrophobicity of the structure and trends are observed between activity, hemolysis, and hydrophobicity.

A primary focus of our computational work in the project has been to evaluate the suitability of a wide variety of backbones, linkers, side chains, and end groups which have been envisioned to be components of the target amphiphilic antibacterial polymers or oligomers. The backbone units for which calculations have been performed to determine lowest energy conformations and torsional potentials include arylamides, salicylamides, aryl hydrazides, aryl oxalamides and mixed backbones containing such sub-units as piperazines, alpha-amino acids, beta-amino acids, pyridines, neo-pentyl glycol, s-triazines, and (CH2)n units.
QSAR (Quantitative Structure and Activity Relationship) models are being developed to correlate experimental antimicrobial activities and selectivities with properties that can be predicted from simulations, such as water-octanol partitioning constants and hydrophobic moments.

ISSUES:
None.

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GRANTING NIH INSTITUTE/CENTER: National Insitute of Biomedical Imaging and Bioengineering (NIBIB) and National Insitute of Neurological Disorders and Stroke (NINDS)

PROJECT TITLE: A 3-D Microfluidic/electronic Neural Interface System: In Vitro Studies of Neural Networks, Plasticity, and Injury

ABSTRACT:
The focus of the proposed program of research is to advance the knowledge of the functionality and the traumatic disruption of neural circuits and networks through the development of a set of technologies that facilitates the in-depth study of three-dimensional (3-D) neuronal tissue in vitro. We are creating a microfabricated neural interface system by combining an array of micromachined towers that incorporate microelectrodes and microfluidic channels. These towers will be fabricated on a substrate that will control and process the signals to and from the towers using integrated circuits. The resulting system will enable a new field of neurobiological research, in which the collective properties of 3-D neural circuits can be observed and manipulated with unprecedented detail and precision, and at a level of control not possible in living animals.

The project has four specific aims, each of which contains a set of technological developments motivated by biological hypotheses:

Specific Aim 1: Fabricate arrays of 3-D microtowers that will support neuronal cell growth and permit integration with microelectrode and microfluidic structures.

Specific Aim 2: Develop a novel multi-site, three-dimensional microfluidic system to locally control the delivery of neural stimuli/nutrients in order to improve cell survival, deliver chemical stimuli, and examine growth and network formation.

Specific Aim 3: Develop custom integrated circuits that will incorporate amplication, multiplexing, and processing of the neuronal data, and will facilitate simultaneous stimulation and recording.

Specific Aim 4: Combine technologies developed in Aims 1-3 (microfabricated towers, microfluidics, and electrical interfacing) with 3-D neural tissue to study information processing, learning, and the morphological and network response to traumatic injury.

Aims 1–3 represent the core elements of the technical development, and have been initiated in parallel during Year 1 of the grant. Aim 4 represents the culmination of the project through the combination of the prior aims, and will be pursued in future years.

The significance of this research lies in the ability to create robust neuronal networks in an in vivo-like cytoarchitecture and precisely study neuronal behavior in normal and injured tissue. Studies resulting from this research will lead to greatly enhanced in vitro investigations and provide technology for the development of "smart" neural implants for replacement of lost sensory and motor function in humans.

STATUS OF RESEARCH AND PARTNERSHIP:
The grant was funded in 2002 August. During the past nine months, progress has been on Aims 1–3.

In Aim 1, three techniques are being developed and compared for the implementation of the micromachined towers using polymer materials. In all three techniques, materials have been tested for biocompatibility and cell viability has been studied using optical imaging of cultured cells on towers.

In Aim 2, stereolitographic rapid prototyping has been used to implement a manifold that can provide uniform nutrient delivery and trophic support to cells. Optical diagnostics have been used to measure velocity and concentration fields on the orifice plate. A tower-based, microfluidic dispensing system consisting of hollow towers and laser-fabricated fluidic ports is also being developed.

In Aim 3, in-house capabilities for fabricating multi-electrode arrays (MEAs) have been used to integrate custom circuitry (chips) onto MEA substrates. To date, the circuitry consists of high-sensitivity, low-noise preamplifiers and multiplexing to record activity of neurons cultured on these integrated systems. Multiple studies of the properties of neuronal-network formation on planar electrode arrays are also in progress.

ISSUES:
There have been no unexpected issues to date.

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung and Blood Institute (NHLBI)

PROJECT TITLE: Rapid Flow Evaluation by Magnetic Resonance Imaging

ABSTRACT:
Velocity encoded cine (VEC) imaging performed using magnetic resonance imaging (MRI) has great clinical potential for diagnosis of cardiovascular diseases. The non-invasive nature of MRI tomographic imaging, its uniform sensitivity to velocity in all directions and its intrinsic 3D nature make it a natural choice for clinical application. Of particular interest is the potential use that can be made of quantitative blood velocity imaging in the assessment of the complex flow fields associated with aortic valvular diseases. Currently, aortic valve diseases are primarily assessed using echocardiography which is widely available, but nevertheless has several important limitations in characterizing flow fields, including views are restricted by the availability of appropriate acoustic windows, results are operator dependant, velocity is detected in only one direction relative to the probe and that primarily 2D views are used to characterize a 3D flow field.
While MR VEC imaging has the potential to provide more comprehensive flow field data than does echocardiography, clinical application of MR VEC imaging has been hampered by its relatively long acquisition times. The powerful gradient systems now available on MRI scanners allow high quality cardiac cine scans to be acquired in comfortable breath-hold times. However, the scan time required for VEC imaging with velocities resolved in 3D is still prohibitively long for most clinical applications.
The goal of this proposal is to implement a rapid MRI approach that has potential to accomplish VEC imaging in a conventional breath-hold time. Development includes MR scanner sequences modification, determining its limits of applicability using computer modeling of flow fields and testing using flow models. In parallel with implementation and validation of the acquisition sequence, processing tools will be developed to analyze the time resolved 3D flow field data sets. Following the development stage, clinical application will be made to patients with aortic valvular diseases.

STATUS OF RESEARCH AND PARTNERSHIP:
We have completed the first year of the proposal. During this year we have accomplished the following:
1) Implemented the BRISK rapid imaging system on our GE CV/i system to quantify flow using the phase velocity method to produce velocity encoded cine (VEC) data sets.
2) Implement data processing routines to construct VEC image sets from BRISK data

3) Developed software to analyze 3D VEC data
4) Investigated the BRISK VEC acquisition technique using computational fluid dynamics (CFD) methods
Implementation of the rapid BRISK imaging sequence has progressed to permit the acquisition of cine data encoding velocity in a though plane manner in a comfortable breath-hold time of 15 s. In preparation for clinical use of rapidly acquired flow VEC data, we have validated the use of MRI VEC data in vivo as being comparable to the data acquired by standard echocardiographic Doppler flow data. This expanded clinical use of VEC data within our team has, in part, been stimulated by the progress of the current flow project. Numerical simulations were conducted for a 3-dimensional viscous internal flow phantom mimicking the human aorta (including ascending, arch and descending sections). Ongoing use of this data is being made to simulate BRISK and variants of it to produce accurate VEC data. Using MATLAB software, processing routines were developed to analyze flow data. The most significant problem was due to the finite slice thickness, which is relatively large with respect to the flowfield features and in-plane pixel dimensions.

ISSUES:
We have not encountered any issues either scientific or administrative that impede the project. While project members are split between three sites, we are able to communicate progress and discuss issues since we have common software platforms which allow data sharing.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB) and National Institute for Neurological Diseases and Stroke (NINDS)

PROJECT TITLE: Bioimaging and Intervention in Neocortical Epilepsy

ABSTRACT:
Magnetic resonance functional and spectroscopic imaging (fMRI, MRS) of the brain provide tremendous opportunities in the study and treatment of epilepsy. In neocortical epilepsy, where the epileptogenic region is highly variable in size, structure and location, deeper insight into the biochemical and functional characteristics of the region and surrounding tissue may provide critical data to assist the neurosurgeon and neurologist in localization and treatment. To fully utilize the multiple forms of available information (MR and EEG), these data must be transformed into a common space and integrated into the intraoperative environment. The work being performed on this grant will develop high resolution MRS and fMRI at 4T and advanced analysis and ntegration methods to better define the epileptogenic tissue and surrounding regions, and enhance our understanding of the biochemical mechanisms underlying the dysfunction in neocortical epilepsy. We will validate these measurements against the gold standard of intracranial electrical recording. These goals will be achieved in this bioengineering research partnership (BRP) by bringing together six partners from 3 academic institutions (Yale (lead institution), Albert Einstein and the University of Minnesota) and 1 industrial partner (BrainLAB, AG) to carry out four integrated programs of scientific investigation and bioengineering development in the area of bioimaging and intervention: 1) development of high resolution fMRI and MRS at 4T for the study of epilepsy; 2) investigation with MRS of the relationship between neuronal damage or loss through the measurement of N-acetylaspartate (NAA), alterations in neurotransmitter metabolism through the measurement of gamma amino butyric acid (GABA) and glutamate, and abnormalities in electrical activity in the epileptogenic region and surrounding tissue; 3) investigation of the relationship between fMRI activation amplitude and the cognitive task, underlying cortical structure, cortical metabolic state, and physiology, and the impact of epilepsy on these factors; 4) development of integration methodologies for fusing multimodal structural and functional (image- and electrode-derived) information for the study and treatment of epilepsy.

STATUS OF RESEARCH AND PARTNERSHIP:
Our efforts are basically proceeding as planned for each of our aims:

1.) Regarding coil design, we have constructed an actively detunable quadrature TEM volume coil. In addition, in our first year we have focused on the development of Dynamic Shim Updating (DSU). Recently, we have focused on implementing these strategies on our 4T human system, which was delivered in September.

2. In Magnetic Resonance Spectroscopy (MRS), we have made significant progress in the following areas: a) we have implemented the N-acetyl aspartate (NAA) 1H spectroscopic imaging sequence and have begun acquiring control and epilepsy patient data; and b) we have developed and implemented a novel glutamate spectroscopic imaging sequence and have acquired control data from normal
subjects.

3.) Regarding functional MRI (fMRI) development, the first year we focused on understanding the effects of motion on fMRI acquisitions. In particular we are currently testing a multi-frame motion estimation algorithm that parameterizes the motion parameters in terms of b-splines. We have also made progress on better quantifying the effects of motion and motion correction on activation detection.

4.) Regarding the image-guided intervention software platform: first, in our efforts to account for brain shift during surgery, we have developed a full continuum finite element model of the linear elastic properties of the brain, and have designed and implemented an initial deformation-compensation system based on data from stereo cameras mounted in the ceiling of the operating room. In addition, we have begun integrating functional image and electrode data into the plafform.

ISSUES:
All partners have been communicating effectively. Several visits from BrainLAB to Yale have helped move along the bridging of software strategies developed at Yale into the image guidance platform, and have alleviated any concerns about an overseas industrial collaboration. In fact, the Yale image processing partner and the BrainLAB partner have completed the design and initial implementation of a research interface for the image guided surgery system.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB)

PROJECT TITLE: In Vivo EPR Bioengineering Research Partnership

ABSTRACT:
Electron Paramagnetic Resonance (EPR) spectroscopy detects unpaired electrons. It is being developed as a tool for monitoring local oxygen concentrations in vivo via the impact of the paramagnetic oxygen on probes with narrow oxygen-dependent lineshapes. To study radicals deep in tissues it is necessary to perform EPR at radiofrequencies where the inherent sensitivity is lower than at the microwave frequencies that are typically used for ex vivo spectroscopy. Much of EPR spectroscopy is performed with magnetic field scans that are slow relative to the linewidth (CW EPR) or by applying pulses of incident radiation (pulsed EPR). There is an intermediate case in which the magnetic field is scanned rapidly through the signal, but it has not been used in EPR because of the need for specialized hardware and the need to process the signal to remove distortions introduced by the rapid scan. However, this approach is expected to be advantageous when dealing with rapidly changing signals and for optimizing scan rate relative to physiological motions. The specific tasks include the design, construction, and testing of an air-core magnet for scanning the magnetic field rapidly. We introduce the innovation that the magnet will be resonated, and magnetic field scans will be sinusoidal. The noise characteristics of the spectrometer and of living samples will be analyzed to optimize scan rates. Software will be written to convert the time axis of the scans to magnetic field axis and to deconvolute the undistorted spectrum from the experimental lineshape.

STATUS OF RESEARCH AND PARTNERSHIP:
In this first year of the project studies were performed to characterize the spin physics of samples that can be used as in vivo probes: lithium phthalocyanine (LiPc) and triarylmethyl (trityl) radicals. Studies with these samples are helping us to define the design specifications for the system that we will build. Small sweep coils were constructed that could be driven with the existing power supplies in the Bruker consoles that we use in our existing 250 MHz and 9.7 GHz spectrometers. With these coils we were able to routinely achieve sweep rates between 10 and 50 kHz and sweep widths of 0.10 to 5.0 G, which encompasses the values that are likely to be useful for the rapid scan experiments.

To have adequate bandwidth, the rapid scan signals were detected through signal paths that normally are used for pulsed experiments, and were detected in quadrature to permit phase adjustment by post-processing. We have written software based on the Bloch equations to simulate the rapid scan signals and find good agreement between experiment and simulation over the full range of sweep widths that have been examined. In the initial validation of the simulation

algorithms, values were known in advance for all of the input parameters. Now that we have confidence in the algorithm, we are using the simulations to predict sweep rates and spectrometer operating parameters that optimize the signal. To maximize signal-to-noise, we also need to characterize the noise in the spectrometer and the noise characteristics for particular types of samples. We have begun these noise studies for the 250 MHz signal detection system.

We have examined a number of key factors that must be taken into consideration in optimizing S/N for a particular class of sample: scan rate, radiofrequency power, and signal filtering. For a test sample of LiPc we have already obtained better S/N per unit time in a rapid scan experiment than in a traditional CW experiment. We have identified a significant 25-MHz clutter problem in the Bruker SpecJet digitizer and we are working to mitigate it via both hardware and software changes. Once this problem is reduced, we should be able to get substantially improved S/N. A number of post-processing techniques also will be examined in the upcoming year. These preliminary results indicate that rapid scan EPR will have importance across many fields of applications.

The collaboration with Bruker has provided hardware at reduced cost and invaluable information concerning their hardware and software. It is already providing input to their design considerations.

ISSUES:

Overall we are pleased with the initial year of this BRP and the useful collaboration between the University of Denver and Bruker BioSpin. When the proposal was submitted in August 2000, we had assembled a highly-qualified team of researchers and established a promising partnership with Bruker BioSpin. There was an unusually long delay between recommendation of funding by the study section and award of funding, due in large part to uncertainties related to the startup of NIBIB. By the time funding began in mid-May, 2002, the engineers at the University of Denver were committed to finishing other projects before they could start on this project, and Bruker had put other R+D higher in priority than this project. However, by the end of the first year, the needed effort has been focused on this project, and Bruker is giving higher priority to data acquisition system development.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Neurological Disorders and Stroke (NINDS)

PROJECT TITLE: Robotically Generated Locomotion in Rodents

ABSTRACT:
The adult mammalian lumbar spinal cord can learn to step in the absence of descending input from the brain. The ability of the spinal cord to learn is an extremely important finding for tens of thousands of spinal cord injured patients, as it could mean the difference between being confined to a wheelchair or being able to stand and take some steps. Understanding how to teach the spinal cord to step through effective rehabilitative training has immediate clinical application in itself and can also play a crucial role in enhancing the efficacy of other potential therapeutic interventions for spinal cord injuries. One method of rehabilitative training, i.e. body weight supported locomotion on a treadmill, has been successful in enhancing locomotor recovery in spinal cord injured animals. There is growing evidence that this form of training can also be used to improve walking in humans that have suffered a stroke or spinal cord injury. The success of training, however, depends on the generation of appropriate patterns of sensory information during weight bearing stepping. We have developed a robotic system to train the hindlimbs of spinally transected rodents to step on a treadmill. The robotic system provides precise control of forces acting on the hindlimbs during stepping and also provides on-line measurement of step cycle trajectory characteristics so that locomotor performance can be quantified quickly and objectively. We hypothesize that the recovery of hindlimb stepping in spinally transcted rats will be enhanced by robotic-controlled locomotor training. We will use the robotic system to control critical training parameters such as the amount of weight bearing on the hindlimbs, the coordination of movements between the two hindlimbs and the amount of assistance provided during training. The first two aims we examine the question of whether providing a maximum amount of weight bearing on the hindlimbs is the most effective weight bearing pattern during training. We will program the robotic device to 1) slowly increase the amount of hindlimb weight bearing within a stepping episode and 2) apply a force field that increases loading by exerting a downward force on the hindpaw. The third aim will examine the effects of imposing different hindlimb coordination patterns on the recovery of stepping. The robotic system will be programmed to train either an alternating gait pattern or an in-phase gait pattern in the hindlimbs. Finally, the fourth aim is to examine the extent that mechanical assistance during training should be provided to facilitate learning by the spinal cord. The recovery of stepping will be compared in rats that receive constant, robotic assistance throughout the step cycle versus rats that receive robotic assistance on an "as-needed" basis. The results of this project will provide a needed behavioral foundation for future research that identifies the specific neurophysiological and molecular mechanisms underlying sensory-enhanced spinal learning. These data will also

provide insight into development of body weight support control and manual (or robotic) intervention for human locomotor training.

STATUS OF RESEARCH AND PARTNERSHIP:
We successfully completed experiments in the first year of the project that address the first specific aim regarding optimal weight bearing patterns. The first task was to develop a new body weight support (BWS) mechanism to control weight bearing. The engineering of the hardware and software was performed in Dr. Reinkensmeyer's laboratory at UC Irvine. The new BWS system uses a spring and a lever arm in a specialized configuration to counterbalance the weight of the rodent. The advantages of the new BWS system are:a reduction in the amount of inertia the animal experiences while stepping, constant force throughout the range of motion of the arm, body weight support can be changed quickly and the amount of support can be monitored and recorded to indicate failure as a function of load bearing. The second task was to implement the weight bearing training algorithms in spinally transected rats. The spinal cord transection surgeries for these experiments were performed in Dr. Edgerton's laboratory at UCLA and the testing of the training algorithms were performed in Dr. de Leon's laboratory at Cal State LA. Three groups of animals were tested: 1) not trained; 2) trained with a fixed body weight support; 3) trained with a ramp body weight support. The key finding of the experiment was that the ramp-trained rats performed more steps than the fixed-trained rats at a range of body weight support levels suggesting that continually challenging the spinal circuits controlling stepping was an effective method for hindlimb training.

ISSUES:
The partnership among the three investigators, Dr. Edgerton, Dr. Reinkensmeyer and Dr. de Leon, has been productive and effective. To date the experiments have proceeded as scheduled. The first experiment is being analyzed and prepared

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB)

PROJECT TITLE: Anti-Inflammatory Coatings for Biomaterials

ABSTRACT:
The prolonged inflammatory response to an implant is one of the primary causes for the failure to integrate into tissue. The two sources of inflammation common to almost all implants are the foreign body response and the relative movement of the implant with the surrounding tissue. Based on evidence in the literature and from our research team, the inflammatory response is mediated by the reactive oxygen species generated by macrophages, leukocytes, and the surrounding connective tissue. Based on our findings, it is evident that titanium dioxide and similar ceramics, even when present as surface coatings of polymeric biomaterials, have the ability to breakdown ROS that have been identified as mediators of the inflammatory response. The goal of this Program is to develop applications for our catalytic antioxidant ceramic technology in the biomaterials and medical device industry. This Program, led by LJBI, consists of five projects with eight academic and industrial partners. Project 1 will investigate the basic mechanisms of action of metal oxides in the catalytic breakdown of ROS. By understanding the fundamental reaction kinetics of the catalytic action of TiO2, catalysts of greater efficiency may be discovered. Project 2 will fabricate and characterize materials for the other four Projects, and partners with Lawrence Livermore National Labs, Drexel University, University of California, Uppsala University, and La Jolla Bioengineering Institute. Project 3 will test the in vivo inflammatory and foreign body response in two in vivo models; a standard rat model and the hamster window model. This project provides a core service to the other projects, but also investigates fundamental mechanisms of the inflammatory response to biomaterials. Project 4 will determine if the catalytic antioxidant ceramic technology is able to mitigate implant-tissue strain-induced inflammation. It will also investigate basic mechanisms of strain-induced inflammation. Project 5 is the interface with the medical device industry. Industrial partners have been chosen to develop applications in different biomaterials areas: Biosensor membranes for implantable glucose sensors (Advanced Tissue and Materials Inc), wound dressing material with anti-inflammatory properties (3M), and dental materials with improved osteointegration (Nobel Biocare). Our overall objective is to provide the proof-of-principle to our industrial partners, which will encourage them to participate in more specific product development.

STATUS OF RESEARCH AND PARTNERSHIP:

The collaboration with our industrial partner Nobel Biocare is progressing well. We are characterizing coatings of titanium dental implants, and correlating the crystalline isoforms of TiO2 to the observed clinical outcomes. We are also investigating whether specific crystalline isoforms of TiO2 promote hydroxyapatite deposition. We have developed an ultra low vacuum RF sputter coating system to accommodate the large size and number of samples that will be processed. Recent results indicate that well-bonded coatings can be produced on silicone surfaces. Macrophage and leukocyte production of reactive oxygen species are now being characterized on coated biomaterials.

ISSUES:
None.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Environmental Health Sciences (NIEHS)

PROJECT TITLE: INTEGRATED SAMPLE PREPARATION FOR GENOMIC ANALYSIS IN MICRODEVICE FORMAT

ABSTRACT:
The overall goal of the proposed research is to produce an integrated sample preparation micro-analytical device for preparing blood samples for genomic analyses. The strategy is to develop a front-end micro sample preparation system, micro-SPS, for use as a research tool with the flexibility to be integrated with a number of downstream genetic analysis platforms, i.e. either sequencing or genotyping. The micro-SPS is composed of three main micro-compartments including: 1.) Sample introduction, combined with cell sorting and selection. 2.) Cell lysis, recovery of the nucleic acid material of choice (e.g. DNA or mRNA), and sample clean up via solid phase extraction or affinity capture. 3.) Elution of the material to an amplification micro-compartment, and subsequent amplification (e.g. via PCR or rtPCR). Studies of the micro-compartment prototypes are paralleled by an investigation of techniques for integrating the micro-compartments into a monolithic and hybrid micro-SPS.

STATUS OF RESEARCH AND PARTNERSHIP:
Aim 1: Sample Introduction, Cell Sorting, Selection and Lysis: Epigenetics refers to the non-genomic information that a cell passes on to its progeny. This ``information'' can vary from methylation of the DNA bases to the occupancy of the promoter and repressor sites on the DNA that control gene expression (see the 293 volume of Science, 10 August for a current look at these issues). In the Austin lab, they are trying to do it all: select rare cells, extract the genomic material, fractionate the genomic DNA and scan the DNA for the occupancy of the epigentic control sites. There have been three major efforts in the Austin lab over the last year aimed directly at rare cell separation: 1. Separation of white cells from whole blood based upon mechanical deformation. The Austin lab has applied extra pressure to this project. They are routinely receiving new blood samples from The Wadsworth Center in Albany NY and Dr. Prinz communicates routinely with Albany. 2. Separation of white cells from whole blood cells based upon a combination of nano/micro magnetics and microhydrodynamics. This project was pursued primarily by M. Shaw and Austin at Princeton. It involves the development of a new separation technology utilizing very small magnetic structures to create very high magnetic field gradients, and ideas that come from our work with R. Huang to create controlled flows in the magnetic structures. My calculations have shown the importance of using relatively thick magnetic films (on the order of 3-5 microns) to achieve the extremely high magnetic field gradients I believe can be achieved using modern magnetic thin film alloys. At the gradients of 103 gauss/micron that I believe we can achieve it should be possible to sort cells based solely on their diamagnetic susceptibility alone. 3. Extraction of genomic DNA from selected cells using a combination of microhydrodynamic flow and dielectrophoretic trapping (covered in the next section, Aim 2).
Aim 2: Recovery of the Nucleic Acid Material, and Sample Clean-up: Miniaturized Solid Phase Extraction: Three years of effort have allowed the Lander'sto demonstrate that on a microchip we can accomplish what we had previously shown possible with DNA using miniaturized capillary systems. Our efforts to define a miniaturized system for solid phase extraction (SPE)-based isolation of DNA on the microliter scale have been successful with two approaches. The first involves exploiting silica beads for SPE of DNA except in a microchip format. This approach was found to be more problematic than in capillary systems because of the small dimensions of the microchannel (relative to capillaries). Flow through the bead bed eventually led to occlusion of the outlet, most likely due to conglomeration of the smaller particles at the outlet. This was solved by essentially "gluing" the beads into place post-packing using a silica-based sol-gel (tetramethylorthosilicate - TMOS). Extraction efficiencies on the order of 60-80% represent the highest reported in the literature to-date for microchip extraction of DNA, surpassing the 50% extraction efficiency reported by Northrup and coworkers using reactive ion etched microdevices.

The Lander's lab has also made progress with eluting the DNA from the sol-gel/bead solid phase using a PCR-friendly buffer. Consequently, it is possible to eluted the DNA is form that is PCR-ready (without further manipulation) so that it will amenable on-chip PCR amplification. The vast majority of the protein is removed in the wash step with little or no protein found in the elution fraction, which contains the majority of the DNA. To show that only the DNA elution fractions contained PCR-ready DNA, a 380 fragment of the beta-globin gene was amplified (off-chip). The fraction at the wash-elution border failed to yield amplified beta-globin DNA, while those fraction well into the elution zone did. This indicates that all of the potential PCR inhibitors present in whole blood have been removed by the process.
The Austin lab is exploring extraction of genomic DNA from selected cells using a combination of microhydrodynamic flow and dielectrophoretic trapping. They have used a sequence of diffusive mixing and electrodeless dielectrophoretic trapping to lyse E. coli cells in a microfabricated environment and trap the E. coli chromosome.characterize the conditions needed for efficient lysis of the cells, the conditions needed to prevent adhesion of the chromatin to the device walls and the dielectrophoretic trapping of the chromatin. This work presents an attempt towards an integrated approach to taking individual E. coli, lysing them on a chip and then holding the chromosomal contents is a dielectrophoretic trap. Lysis of the cells was achieved by rapid diffusional mixing of low ionic strength water with an osmotically stressed cell (a spheroblast). In the mixing region the spheroblast is brought in using hydrodynamic flow from below, while the low ionic strength buffer comes in from the left. At the T junction the cells lyse due to the positive osmotic pressure of the cell contents. Once the cell is lysed we then transport the lysed cell contents into a region where the chromosome of the cell can be separated from the other components of the cell, as shown. They utilize the technology of dielectrophoretic trapping at low frequencies to separate the chromosome from the lysis debris. Cells flow under hydrodynamic flow from the cell entry region to mixing region 1 where they are lysed. Mixing region 2, not used in this experiment, can be used for further clean-up such as protein hydrolysis or buffer change. Either hydrodynamic flow or DC electric fields can be used to transport the genomic material to the dielectrophoretic trap region. A simultaneously applied AC electric field can be used to trap the genomic material in the electrode array.
Aim 3: Amplification of Nucleic Acid Material:
We have developed infrared (IR)-mediated PCR for on-chip amplification of DNA with the goal in year five that this on-chip process with be flanked by SPE of DNA (upstream) and separation of detection (downstream). Interfacing the PCR amplification of some specified volume of PCR mixture (currently ~1-2 μL) with on-chip analysis is not trivial owing to the specific needs of the microstructures used for these very disparate processes. While the surfaces the PCR chamber (to prevent Taq adsorption) and the separation domain (to minimize electroosmotic flow) have to be coated, the same coatng agent does not suffice for both. We have solved this technical problem by coating the PCR chamber with epoxydimethylacrylamide and the separation domain with a adsorptive seiving we have recently developed. Having accomplished that and shown that these are optimal for their specific processes, we have been been able to demonstrate the first on-chip IR-PCR amplification linked directly to separation and detection. The amplification is completed in ~500 seconds, representing roughly an order of magnitude inprovement amplification speed. Injection was very lengthy, requiring almost as much time as that needed for the PCR. This was a design problem (distance between the PCR chamber and separation channel too large) that was corrected with the next set of prototypes. Separation was as fast as expected for a microchip-based electrophoresis, completed in a few hundred seconds. This demonstration, we believe, represents a milestone, since it shows the ability to PCR amplify and genotype on the same chip – once cell sorting a DNA extraction (discussed in the previous section) have been integrated, microchip-based sample processing can be realized. If the chip is designed to minimize the distance between the PCR chamber and separation channel, the injection time is reduced to ~60 sec, which is much more reasonable, allowing for injection and separation to be complete in a few hundred seconds. The amplification shown here is for anthrax and required an extensive number of cycles in order to ensure anplification. As shown, the amplification was successful with a sufficient amount of the anthrax DNA being amplified for detection on a microchip.
Aim 4: Integration of Independent Prototype Micro Compartments into a Hybrid micro-SPS:
The Frazier lab has been coordinating integration of the micro-SPS compartments. During year three, the BRP team has chosen technology pathways to realize both a hybrid multi-chip micro-SPS and a monolithic micro-SPS. The hybrid system consists of two glass based microfluidic chips with interconnects between the chips. Stereolithography and capillary tubing are used for the on-chip fluid interconnects required for integrated actuators (e.g., pumps, valves), and for fluid pathways between chips. The monolithic micro-SPS is fabricated using a combination of plastics, glass, and perhaps silicon for on-chip functionality. The monolithic system utilizes latex and PDMS valves to control fluid flow. Micro stenciling is used for patterning of metal conductors, detectors, and inner-layer electrical vias. As with the hybrid approach, stereolithography is used to realize the fluid interconnects and fluid pathways. For the monolithic SPS, layer-to-layer bonding will be a critical factor in system reliability.

ISSUES:
Primary issues are the logistics of integrating the technologies developed from the participating laboratories into a common format for the total analysis system. We have addressed this issue by adding a 'roving' research scientist into the program to aid with dissemination of the base fabrication technologies developed at GaTech to the other sites as well as to lead prototyping efforts of devices using the base fabrication technologies.

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung and Blood Institute (NHLBI)

PROJECT TITLE: Micromechanics of airway smooth muscle cells in culture

ABSTRACT:
Acute narrowing of the airway lumen in asthma is driven by myosin motors that exert their mechanical effects within a cytoskeletal scaffolding that is both deformable and in a continuous state of remodeling. The mechanical properties of that scaffolding are not well defined. This BRP grant is a multi-discliplinary design-directed bioengineering project that is intended to fill that gap of knowledge. We have developed a micro-nano scale mechanical technology to measure the rheological properties of adherent living airway smooth muscle cells in culture, and the time-course of mechanical changes that occur in response to contractile stimuli or after genetic manipulation of cytoskeletal proteins.

Ligand-coated ferromagnetic microbeads are bound to the cytoskeleton, and oscillatory mechanical torques are then applied to the bead by a sinusoidally-varying external magnetic field. Resulting oscillatory bead motions deform the cell, and can be determined by measuring changes of the remanent magnetic field due to bead rotations or, alternatively, by direct observation of oscillatory bead displacements using light microscopy; these are complementary detection methods each with special advantages. This technology becomes, in effect, a micro-rheometry system that can probe – in cell culture conditions – contractile responses and underlying cellular rate processes over time scales as short as milliseconds to as long as hundreds of seconds, and with deformations as big as 500 nm and as small as 5 nm. Thus, it measures mechanical properties of cells using deformation times and deformation magnitudes that span the physiological range. We are currently refining the technology and, as a proof of concept, using it to test the hypothesis that the contractile response of human airway smooth muscle cells in culture is attenuated by overexpression of heat shock protein 27 (HSP27) dominant negative mutants. This hypothesis bears upon a question whose importance has been identified only recently, namely, the stability of the cytoskeleton of the airway smooth muscle cell and the role of CSK stability in airway narrowing in asthma.

STATUS OF RESEARCH AND PARTNERSHIP:
Research is progressing on schedule and several publications have appeared already in the literature. Using magnetic bead twisting over a wide range of oscillatory frequencies we have established a remarkable glass-like behavior of the cytoskeleton, and shown that this finding is confirmed when the cells are probed by an independent method, atomic force microscopy. So far, the BRP partners have met for six two-day meetings that have been intense, mutually beneficial and highly productive.

In addition, Dr. Ben Fabry, who was a postdoctoral fellow and then a Research Associate working on this project, is moving to the University of Erlangen, Germany, where he has been appointed Professor of Bioengineering. At his new post, he will remain a member of this partnership.

ISSUES:

The issues that we have encountered are all highly positive. The BRP granting mechanism has allowed us to pursue avenues of investigation and to facilitate important collaborations that would otherwise have been most difficult to accomplish.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)

PROJECT TITLE: Role of Biopolymers and Lipids in Kidney Stone Formation

ABSTRACT:
The objective of the proposed bioengineering research partnership (BRP), located at the University of Florida, is to examine two key issues relevant to urolithiasis; 1) the effects of acidic biopolymers and lipid membranes on nucleation, growth, and aggregation of calcium oxalate (CaOx) crystals in an artificial urinary environment; and 2) the injurious effects of a liquid-phase mineral precursor on tubular epithelial cells grown in culture. The long-range clinical goal of this BRP is to provide a more effective means of diagnosis, treatment, and long-term prevention of renal calculi. The following 4 topical areas are being addressed:

STATUS OF RESEARCH AND PARTNERSHIP:
I) Crystal-Macromolecule Interactions: The Specific Aims for this topic area deal with the effects of acidic proteins and mimetic peptides on crystal nucleation and growth, with emphasis on the ability to generate the polymer-induced liquid-precursor (PILP) process of mineralization in either the calcium oxalate or phosphate systems, both of which are relevant to stone formation. As opposed to the calcium carbonate system, a combination of polyanionic additives has proved most successful for generating CaP PILP phase.
Statistically designed experiments are being used to examine the effect of various factors on nucleation & crystal growth of calcium oxalate monohydrate (initially without the PILP mechanism). This past year focused on the effect of some urinary species such as citrate, protein, and oxalate, on the nucleation & crystallization characteristics of COM.
II) Crystal-Crystal Interactions: Because stones frequently contain a calcium phosphate spherulitic core, the heterogeneous nucleation of Calcium Oxalate Monohydrate (COM) on Amorphous Calcium Phosphate (ACP) droplets formed via the PILP process was examined. This was done by a vapor diffusion technique to first introduce the phosphate counterion into a calcium containing solution, and then the oxalate counterion for the overgrowth. The COM crystals overgrown on ACP in the presence of polymers produce mushroom-shaped spherulites that are not seen in the homogeneous nucleation of COM, which mainly produces single-crystalline morphologies. This suggests that ACP not only serves as a template for crystal growth of COM, but also changes its growth behavior, producing a composite structure consistent with the structures found in mixed urinary stones.

IV) Crystal-Cell Interactions: The objective of this work is to examine the interactions between crystals and relevant renal epithelial cells. Force/distance profiles have been measured for the interaction of the AFM tip or COM crystal with LLCPK1 and MDCK cells in artificial urine solutions, as well as in albumin/oxalate/

citrate containing solutions. For COM crystal/cell interactions, both repulsive and adhesive forces are observed. Albumin inhibits the attachment of the COM crystals to the cell, while oxalate ions promote the process.
It has been suggested that Br crystals materializing in the late proximal tubules or loops of Henle can promote nucleation of CaOx in the collecting ducts. We have been investigating the renal epithelial cellular response to the presence of calcium phosphate crystals. Exposure of both LLC-PK1 and MDCK cells to Brushite (BR) crystals resulted in significant increase in the release of LDH into the culture medium, indicating the Br crystals are injurious to renal epithelial cells and that lipid peroxidation is involved. Differences are seen between the two cell types. Recent studies have also shown that retention of CaOx crystals is promoted by injury to the renal epithelium. Based on data presented here we propose that CaP crystals may not only nucleate CaOx crystals but also promote their retention in the kidneys by injuring the renal epithelium.
III) Crystal-Lipid Interactions: Using the Brewster angle microscopy (BAM), we are characterizing calcium oxalate crystallization at phospholipid monolayers. BAM can be used to determine where COM precipitates in a mixed monolayer spread over a calcium oxalate subphase, in which the lipids mimic the membranes of cells or cellular degradation products. Studies of two phase monolayers with either gas and liquid-expanded, or liquid-expanded and liquid-condensed phases present, show preferential crystal growth at phase boundaries. In contrast to these experiments, where a single phospholipid is in equilibrium between phases, phase-separated binary phospholipid mixtures were also studied. Crystal growth in this case is confined to domains of DPPC and is not observed at phase boundaries.
An Optical Trap Force Transducer is being used to measure and characterize interactions between single calcium phosphate PILP particles and phospholipid bilayers of different compositions. To reach this objective, we first had to advance the optical trap/evanescent wave light scattering force measurement technique to measure particle-surface interaction forces against solid-supported phospholipid bilayers created using the Langmuir-Blodgett technique in situ. Then PILP-surface interactions were measured under conditions where the repulsive electrostatic forces acting on the particle were expected to be dominant and the viability of the technique for these particles could be shown. Extending on our successful measurements on supported bilayers and on PILP particles, we are presently attempting to measure the combined PILP particle-phospholipid bilayer system as a function of phospholipid composition and $CaCl_2$ concentration.

ISSUES:
None.

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GRANTING NIH INSTITUTE/CENTER: National Eye Institute (NEI)

PROJECT TITLE: Development/Testing of Artificial Retinas for the Blind

ABSTRACT:
Our research for this partnership grant is to develop a long-term implantable retinal stimulator for patients blinded by outer retinal degenerations. Using technologies developed by the Alfred E. Mann group of companies over the past 30 years for implantable stimulators, we are developing a chronic retinal stimulator and associated external hardware for use both in research and as a clinical device.

In order to achieve this goal, several areas of research are still needed. In this bioengineering research partnership, academia collaborates with industry to accomplish the basic research necessary to make a chronic retinal prosthesis a reality. Areas of basic research that we focus on include:

Electrode geometry and electrode material selection
Surgical attachment of the retinal implant
Low power electronic circuit design
Hermetic packaging

Each of these areas needs additional research for the creation of an optimal chronic retinal prosthesis which will enable persons blinded by outer retinal degenerations to regain the most important loss they have suffered--the loss of mobility. The aim of this five-year proposal is to complete the design and manufacture of a retinal prosthesis and associated external hardware and test it chronically in animals, so that an investigational device application can be made to the FDA in preparation for a clinical trial.

STATUS OF RESEARCH AND PARTNERSHIP:
The partnership continues to make exceptional progress in its third year.

The new material from last year still functions much better than the prior industry standard, platinum, and has been under test for eighteen months so far. This new material will allow much denser electrode arrays which will hopefully provide finer resolution for a retinal prosthesis. We plan to use this material in a next generation device. A second material has also been identified which so far performs even better than the first in terms of maximum charge density.

Chronic animal studies in dogs with wireless implants are still underway at USC. Over 25 active wireless implants have been implanted to date. In the histology examined so far, electrical stimulation does not appear to have affected any of the retinal cells underneath the arrays for periods of up to three months of stimulation. Longer periods of stimulation are under test.

In related privately funded work, patient tests of a Model 1 implant are underway signifying the achievement of the major goal of the first phase of the partnership.

More advanced devices with higher electrode counts are still under development. Testing of the fifth generation integrated circuit (the 'brain' of the implant) is complete and it is functioning well. Development of packaging and electrodes for these advanced devices also continues to advance with several approaches under test.

ISSUES:
Despite various efforts, distance and communication continue to be challenging for partnership members outside of our home state of California. The relocation of the entire Johns Hopkins team to USC has helped the project immensely. Wentai Liu from North Carolina State has agreed to move to California as well. Future plans include consolidating other partnership members to physically closer locations.

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung and Blood Institute (NHLBI)

PROJECT TITLE: Magnetic Resonance Guided Electrophysiology Intervention

ABSTRACT:
Ventricular tachyarrhythmias and atrial fibrillation are the most important arrhythmias affecting patients. They are the most frequently encountered tachycardias, account for the most morbidity and mortality, and despite much progress, remain therapeutic challenges. Invasive electrical studies of the heart (electrophysiologic studies) are often used in the diagnosis and therapy of arrhythmias, and many arrhythmias can be cured by selective destruction of critical electrical pathways with radiofrequency (RF) catheter ablation. A major limitation in studying arrhythmias in patients, however, is the lack of ability to accurately correlate anatomical and electrical information. Anatomy is derived from x-ray images, which are two-dimensional and have substantial anatomic ambiguity. Another major limitation is the lack of ability to visualize ablated areas of myocardium during catheter ablation procedures, making it difficult to confirm the presence of ablated lesions in the desired locations. We have developed ways of combining the anatomic information from magnetic resonance imaging (MRI), with electrophysiologic testing and catheter ablation.

We hypothesize that magnetic resonance imaging, with transesophageal receivers, intracardiac receivers and MRI-compatible (non-magnetic) electrode catheters, can (1) provide accurate navigation of catheters without radiation, (2) provide the ability to visualize ablated lesions, and (3) aid in producing more accurate electrical maps. As a prototype for the development of new approaches to electrophysiologic testing and catheter ablation, this proposal addresses atrial fibrillation primarily. The imaging technologies developed in this project, should however, be broadly applicable to using MRI to guide interventional procedures in the heart in general, as well as in other organ systems.

STATUS OF RESEARCH AND PARTNERSHIP:
We have added a partner, Mico Helix Incorporated, which will be developing miniature coils for incorporation into the catheters, to protect them from high intensity MRI radio-frequency fields. Our Investigational Device Exemption (IDE) for MR-guided studies and in patients has been completely revised and resubmitted.

We have obtained improved catheter tip location sensors. These sensors are less than 1.2 mm in diameter. They have been validated in the MR scanner and it has been determined that they provide position accuracy to plus or minus 1 mm. This technology, along with a custom control console, will allow dynamic manipulation of the imaging plane to keep it in the plane of the catheter tip.

We have developed 3 D imaging software. The data from the catheter tip location system is being interfaced with the 3 D software to allow display of the catheter tip position and direction on the 3 D images of the heart.

We have demonstrated that real time positioning of catheters is feasible using MR guidance alone. This ability is critical to the success of MR guided catheter based interventions. We studied dogs where the MRI-compatible catheters were introduced into positions normally used in routine EP studies using real time (5 frames/ sec) MRI sequences. Catheters were manipulated until standard intracardiac electrogram signals were obtained. These studies also showed that no significant distortion of the intracardiac electrogram is generated by MR imaging – another critical step in demonstrating the feasibility of MR guided interventions.

ISSUES:
We have added and deleted partners, and are pleased with the flexibility of the BRP, as these changes have enhanced the overall program.

We would like to know what criteria would be used for BRP renewals.

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GRANTING NIH INSTITUTE/CENTER: National Institute for Biomedical Imaging and Bioengineering (NIBIB)

PROJECT TITLE: Imaging Structure and Function in Small Animals

ABSTRACT:
This bioengineering research partnership will develop a dual-modality CT/SPECT system for high-resolution imaging of radionuclides in transgenic and knockout mice that now are in widespread use to model the mechanism, diagnosis, and treatment of human diseases. This research will focus on the development of techniques that correlate structure and function, and that can perform noninvasive and quantitatively accurate measurement of tissue metabolism and organ physiology in small animals using radiolabeled tracers. The research program includes 5 specific aims. (1) A pinhole SPECT system will be developed for radionuclide imaging of small animals. Two interchangeable detector arrays will be developed, one for imaging low-energy radionuclides such as 125I (27.5 keV), and the other for imaging 99mTc (140 keV) and other radionuclides having higher photon energies. (2) The pinhole SPECT system will be integrated with a cone-beam computed tomography system volume to allow sequential acquisitions of CT and SPECT images without moving the animal. (3) Cone-beam tomographic algorithms will be implemented for reconstructing the radionuclide and x-ray tomographic data from the small animal imager. Techniques will be developed that use the reconstructed CT and SPECT data to quantify regional distribution of radionuclide concentration at spatial resolutions suitable for mice. (4) The dual-modality imaging system will be used for in vivo measurement of cardiovascular physiology in transgenic mice to investigate the role of the sympathetic innervation in heart disease. (5) The dual-modality imaging system will be used to measure the tumor and organ distribution of humanized anti-HER2 monoclonal antibody in a transgenic mouse model of metastatic breast cancer. The overall goal of this project will develop a high-resolution imaging system that combines CT and SPECT to correlating structure and function. The system also will be designed to perform noninvasive serial studies in mice, and to replace invasive direct tissue sampling and autoradiography for biodistribution studies and functional assessments using radiolabeled tracers in transgenic mice.

STATUS OF RESEARCH AND PARTNERSHIP:
We have just completed Year 2 of our 5 year project to develop a combined SPECT/CT small animal imaging system. In Year 1, our partnership developed conceptual designs of the proposed small animal imaging system. In Year 2, we began the detailed design of the imaging system

gantry (lead by Jamco Engineering), have completed design and are implementing the system control software and electronics (led by UCSF), and now are completing assembly and beginning bench-tests of the CT subsystem (led by UCSF) and the radionuclide detector and read-out subsystem (led by Photon Imaging Inc.). At UCSF, we also are developing and refining image reconstruction and correction software that uses the correlated CT data to refine the visual quality and the quantitative accuracy of the radionuclide data obtained with SPECT. Our goal for the next year is to complete system development and integration, and to begin system evaluation and phantom imaging as a prelude to in vivo imaging with small animals. Our partnership is unchanged from our original proposal. The primary effort during Years 1-2 have been provided by the engineering partners, UCSF Physics Research Laboratory, Photon Imaging Incorporated, and Jamco Engineering. As the program proceeds, then we will increase the participation of Genentech Incorporated and UCSF collaborator, Michael Dae, M.D. Dr. Harrison Barrett at the University of Arizona will be consulted to provide expertise and to review the project.

ISSUES:
This project will develop high-resolution dual-modality imaging system for imaging small animals. There are two major issues which have affected our program over the past two years.

1. Originally we planned to configure a radionuclide detector for this system that would incorporate a silicon photodiode array and cesium iodide scintillator. We how are planning to use a room-temperature semiconductor detector (cadmium zinc telluride). This technological change has presented engineering challenges which we and our partner, Photon Imaging, are working to overcome. However, the solid state detector will achieve energy resolution and better performance for low-energy radionuclides such as iodine-125 that are important for small animal imaging.

2. No small animal dual-modality imaging systems had been developed when we first proposed this work. Now several groups, including the University of Arizona, Johns Hopkins University, and the University of Virginia, have developed small animal SPECT/CT systems, and University of California, Davis, is developing a small animal PET/CT system. Nevertheless, we believe that the small animal SPECT/CT system being developed within our BRP will have unique capabilities that are not available elsewhere, and our overall goals remain the same. In addition, under SBIR funding, Photon Imaging (assisted by our group at UCSF) has developed a small animal SPECT/CT imaging system. This system now is available commercially, and provides a platform from which we can gain experience and gather data to assist us with the development of the new imaging system being developed under our BRP.

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute (NHLBI)

PROJECT TITLE: Total Liquid Ventilation: A Bioengineering Partnership

ABSTRACT:
ARDS is a frequently lethal pulmonary process that occurs in approximately 150,000 patients each year. Total liquid ventilation (TLV), in which the lungs are filled with perfluorocarbon and ventilated with a device which oxygenates and removes carbon dioxide from the perfluorocarbon, has great potential to effectively treat patients with ARDS. The clinical principal investigator has been performing studies in liquid ventilation over the last 8 years. Through our laboratory effort, we have generated data that demonstrate the efficacy of TLV in improving gas exchange, pulmonary function, and oxygen delivery, as well as in reducing acute lung injury. The bioengineering principal investigator has been performing studies in biofluid mechanics and transport of the pulmonary system for many years. This proposal addresses several fundamental physiological and bioengineering issues that underlie the progress toward establishing TLV as a clinical tool: 1) the optimal means for administering the liquid into the lungs; 2) the effect of ventilation parameters upon gas exchange; and 3) the expiratory flow limitation which restricts the effectiveness of the technique. The current research proposal is, therefore, directed at developing a new partnership between a clinician scientist and a bioengineer in the investigation of these issues which involve principles of fluid delivery and distribution, gas transport, and flow limitation during expiration. Specifically, our investigation will assess the distribution of the perfluorocarbon with regard to rate of fill, position during filling, and the characteristics of the perfluorocarbon. Secondly, we intend to investigate and to model the parameters which affect gas exchange during TLV, such as tidal volume, respiratory rate, and lung distension, and to model local flow patterns within the airways and alveoli. Finally, we plan to assess the relationship of flow limitation during expiration to the rate of flow and the state of inflation of the lungs and to investigate strategic means of manipulating parameters which determine flow limitation. A thorough understanding of these issues and solutions to these problems will be critical to the clinical application of this new and exciting technology.

STATUS OF RESEARCH AND PARTNERSHIP:
Our research in the above project has progressed well. We have developed data evaluating the effect of rate of perfluorocarbon administration and position with respect to gravity upon the homogeneity of the distribution of perfluorocarbon in the lungs. We have also characterized the effect of perfluorocarbon flow rate and lung volume upon the development of flow limitation and have defined predictors of the onset of flow limitation which will allow servoregulation of liquid drainage from the lungs and avoidance of airway collapse. We have examined the various

properties of perfluorocarbons which are associated with the development of flow limitation. We are now studying the effects of bronchodilation upon airway resistance and flow limitation. Our group has developed models of gas exchange in the alveolus partially filled with perfluorocarbon. We have assessed the relationship of ventilatory parameters in-vivo during TLV to gas exchange and are using these data to model alveolar gas exchange in the totally perfluorocarbon-filled lung. Finally, we are developing and testing a variety of devices to perform TLV.

ISSUES:
We hold conferences involving all members of the partnership, along with trainees, every 2 to 4 weeks. Ongoing research is discussed and data presented at these meetings with vital input contributed from both the bioengineering and clinical investigators. These conferences, with the associated integration of expertise, have resulted in a broader approach to our research.

We have numerous trainees involved in the partnership including two M.D. research fellows, one medical student, and two bioengineering post-doctoral students. One of the bioengineering junior faculty, who was a previous minority supplement post-doctoral trainee on this grant, spends a significant portion of his time in the animal laboratories of the clinical partner, learning to perform animal experiments and to apply biologic data to his research. Together, all of these trainees have been critical to sustaining the cross-fertilization and integration that has been valuable to this partnership.

We have no specific problems with the partnership.

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute (NHLBI)

PROJECT TITLE: Image and Model Based Analysis of Lung Disease

ABSTRACT:
This BRP is a consortium of researcher from 6 Universities joined together to develop methods associated with high speed volumetric x-ray CT imaging to study lung structure and function and to use those measures to build an atlas/model of the normal male and female lung for 3 decades of adult age ranges with the notion that this atlas/model can then serve as the standard against which the individual can be compared for early detection, quantification and tracking of lung pathology. As the core of our research efforts, we have established a research dedicated multi-detector row CT scanning facility and image analysis facility at the University of Iowa in partnership with Philips Medical Systems.

STATUS OF RESEARCH AND PARTNERSHIP:
We have had significant successes this grant period. We have added a new partner with the awarding of a supplement to our BRP. Michael Hlastalla, Tom Robertson and Melissa Krueger from the University of Washington have joined our team with unique expertise in the use of the Multiple Inert Gas Elimination Technique and a method for potentially extracting both ventilation and perfusion from the CT-based xenon wash-in signal. The techniques for functional lung imaging developed in this BRP have allowed our colleagues at Johns Hopkins (lead by Brett Simon) to successfully extend our work into other aspects of human lung disease. Dr. Simon and colleagues have received funding from the Department of Defense to use the BRP methods for an animal study of exogenous surfactant use in acute lung injury (DAMD 17-02-1-0732, PI B. Simon) with a consortium arrangement established with Dr. Hoffman and the core scanning facility at the University of Iowa. In addition, BRP technology plays an important role in the recently funded NIH Acute Lung Injury SCOR program at Johns Hopkins. Through an extension of our lung imaging methodologies for use in micro-CT imaging of mice to study mouse models of genetic-based lung disease, Drs Wang, Hoffman and McLennan have successfully competed for an R21/R33 Grant from the NIH (R21EB001685: Development and Integration of Bioluminescent CT) to establish a combined bioluminescent CT-micro X-ray CT scanner for the tracing of the link between vector target activity and structural and functional responses. The core lung imaging software technology has reached a state where it is sought by commercial partners, and we have applied for an SBIR grant to utilize our histogram-based analysis software along with our airway segmentation software to provide an intrabronchial roadmap to the lung regions

targeted for lung volume reduction so that appropriately sized intra-bronchial one way valves can be placed as an alternative to lung volume reduction surgery. We have added Dr. Ananth Annapragada, recently from Cleveland State University and now from the University of Texas in Houston. Dr. Annapragada is a chemical engineer who has worked with our team to design and test a new class of x-ray blood pool contrast agents based upon nano-scale stealth liposome technology. We have demonstrated in Rabbits that we are able to inject these agents into the blood pool, achieve a 150 Houndsfield unit enhancement of x-ray attenuation and the enhancement was sustained for the full 3.5 hours of observation of the living, anesthetized rabbit when scanned on via our multi-detector spiral CT scanner. This is shown in figure 1 and the work recently appeared in Academic Radiology. A unique feature of this class of contrast agents is that they are expected to leak into extravascular spaces in tissues undergoing angiogenesis (ie: cancers) and to not return to the vascular space, thus selectively enhancing tumors. These agents an be tagged with antibodies for selective targeting of tissues, and we expect that this approach to blood pool imaging will be of significant help in evaluating the presence and time course of pulmonary emboli. This enhancement will aid considerably in our efforts to segment the pulmonary vascular tree.

We have established a collaboration with Dr. Merryn Tawhai and Peter Hunter from the Bioengineering Institute in Auckland, New Zealand to extend our efforts to build a volumetric model of the lung. Dr. Tawhai is utilizing both human and sheep lung images to generate specific airway and vascular models which have as their boundary conditions the boarders of the chest wall segmented from specific volumetric image data sets made available to her from our BRP-derived studies.

We have linked forces with the Hydraulics Institute here at the University of Iowa to begin to develop a CFD model of gas flow within the bronchial tree to help us understand recently observed differences in washin and washout measures using Xenon gas as a radiodense tag of gas flow.

Our paper in which we reported on our evaluation of Xenon CT for use in assessing regional ventilation parameters (Tajik JK, Chon D, Won C, Tran BQ, Hoffman EA. Subsecond multisection CT of regional pulmonary ventilation Acad Radiol 2002, 9(2): 130-146.) won the Association of University Radiologist's Herbert M. Stauffer, Outstanding Basic Science Paper.

ISSUES:

None.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Dental and Craniofacial Research (NIDCR)

PROJECT TITLE: Engineering Joint Scaffolds for Concurrent Function and Regeneration

ABSTRACT:
Tissue engineering offers considerable promise for temporomandibular (TMJ) joint reconstruction, a pressing clinical problem. To create durable engineered joint implants, the effects of scaffold material and architecture on tissue regeneration and function must be understood. To fill this vital need, we must be able to systematically study controlled scaffold architecture effects on bone regeneration, bone-cartilage regeneration, and load bearing capability. In this BRP, we will determine the effects of designed and fabricated internal architectures on bone regeneration by bone marrow stromal cells in an in vivo model of osteogenesis. We will mechanically test these architectures to determine load carrying capability. To test bone-cartilage interface regeneration in vivo, we will create a scaffold interface design seeded with bone marrow stromal cells on one half of the scaffold (bone side) and auricular chondrocytes on the other half (cartilage side), creating a bone-cartilage interface inside the scaffold. Finally, we will then engineer a prototype Conylar Ramus Unit (CRU) based on the most promising data from the bone-bone and bone-cartilage scaffold studies. The primary goals of this BRP are to:

1) Determine how two scaffold materials (hydroxyapatite (HA) and polyanhydride and four porosity variations within controlled architectures affect bone regeneration and load carrying capability.
2) Determine how scaffold interface designs using HA and polyanhydride for the bone half and polyanhydride and PGA for the cartilage half affect bone-cartilage interface regeneration
3) Test one prototype CRU scaffold that incorporates the best results from 1 and 2 in an in vivo minipig model at 3, 6 and 12 months. The prototype CRU will have designed external shape and scaffold architecture.

Our first two specific aims are to apply image-based optimal design and solid free-form fabrication to create the scaffolds. The remaining four specific aims are to investigate the performance of these scaffolds mechanically and using subcutaneous models, resulting in the in vivo minipig test of a prototype CRU.

STATUS OF RESEARCH AND PARTNERSHIP:

We have made significant progress on both aims one and two during the past year. We have modified the aims, however, to focus on three porosity levels (30%, 50%, and 70%) but have expanded scaffold materials to include Tri-Calcium Phosphate (TCP), poly-L-lactic acid (PLLA), poly-lactic-glycolic acid (PLGA) and HA/PLLA composites. The first specific aim involves computational design and fabrication of scaffolds in addition to mechanical testing. Based on specific aim 1, we have published two papers on scaffold design and fabrication, another paper in press, with another paper submitted. The papers on computational design showed that we can design both polymer and ceramic scaffold architectures to match mandibular condyle bone samples. We have also demonstrated the capability to manufacture HA, HA/TCP, PLLA, PLGA and PGA scaffolds and test their mechanical properties. For PLGA, we demonstrated stiffness ranging from 112 MPa (70% porous) to 375 MPa (50% porous) and ultimate stress ranging from 1 to 14 MPa, well within the range of bone. For specific aim 2, we have a paper showing generation of a bone cartilage interface on a discrete HA/PLLA scaffold that was presented at the biomaterials meeting. We have also demonstrated the capability to pulse bone marrow stromal cells with chondrogenic media and make cartilage like tissue. We are now investigating how different carriers affect cartilage regeneraton. All told, we have presented 5 abstracts at meetings this year related to the work. We are also expanding the work on interfaces to utilize bioreactors with our Georgia Tech partners.

ISSUES:

Getting good integration with partners is the major issue with our partnership. We have begun to address this with the PI visiting Georgia Tech to begin the bioreactor portions of the study. We will do the same with our Colorado partner. Otherwise, we feel that progress is good. We have begun utilizing a web based resource for partner integration.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB)

PROJECT TITLE: Quantitation of Cellular Protein Production in Real Time

ABSTRACT:
The study of individual cells and mixed populations of cells provides a powerful approach to unraveling the simultaneous effects of paracrine, juxtacrine, and matrix-associated factors in the poorly understood cellular processes that underlie normal and disease processes such as osteoblast differentiation and age-related bone loss. In many cases, progress in the purification of progenitor cells and rare stem cells has outpaced development of technologies for gathering and interpreting information at the individual cell level. Our biomedical research partnership combines the technological expertise of Automated Cell, Inc. (ACI) with the biomedical expertise of researchers at University of Pittsburgh Cancer Institute (UPCI), Carnegie Mellon University (CMU) and Harvard Medical School (HMS) to focus on the development of tools for real time study of individual cells and mixed populations of cells in an automated combinatorial cell culture system.

Goals include: 1) Development of software for real time analysis and dynamic manipulation of osteoblast progenitor/precursor cells; 2) Development of devices and procedures for coherent real time detection of phenotypic changes in rare human cells and the progeny of such cells deposited, tracked, imaged and analyzed individually through time; 3) Innovation of technologies for real time quantitation of cellular protein production through miniaturized assay methods and off-line analytical systems; and 4) Application of these technologies to determine effects of aging on osteoblast differentiation potential of human bone marrow stromal cells, utilizing the subset, STRO-1+, in an in vitro model system.

STATUS OF RESEARCH AND PARTNERSHIP:
Software for experimental setup in 384 well plates has been improved through: 1) integrating cell culture plate information from the stage of experiment planning to final data analysis by linking plate locations and treatment specifications automatically through a database to the various down-stream programs, and 2) implementing a subprogram to perform autofocus at each location in the plate such that images are optimized for specific characteristics providing the most reliable data from our down-stream image-processing software.

We have constructed a fluidics-development station as proposed that incorporates an automated stage and a specially constructed bio-environmental chamber with a mirror and

transparent top for horizontal and above-well viewing. A low-resolution camera and inverted microscope beneath the needle captures images and video during execution of procedures. Improved user-interfacing software has been designed for operation of the various valves, syringes, and needles employed in the fluidics devices and for integration of these components into macros involving the automated stage movement. The first application of the fluidics station has focused on successful development of a proprietary method for immuno-fluorescent staining with low background and without disruption of cells in 384 well plates. The method will be applicable to in situ microbead analysis for secreted protein and for periodic cell surface marker analysis during the process of osteoblast differentiation.

The partnership has tested osteoblastic cells for effects of the staining media and has begun to characterize the effect of different extracellular matrices on the growth and behavior of these cells as proposed. Significant biological changes (e.g. 2 to 3-fold increased motility rate and increased cell scattering) were demonstrated in a dose-dependent manner for cells grown in the presence of laminin versus collagen or uncoated plastic. Development of statistical methods for analysis of complex multiparametric interactions of the cell culture environment has involved collaborators from HMS, ACI, and UPCI.

ISSUES:

In less than one year since initiation of this project, we are well underway with successful development and demonstration of an innovative method for routine assay of phenotypic changes in individual cells grown under continuous monitoring in 384 well plates. The collaboration is working well with technology and methods development occurring at the ACI facilities and with biological monitoring and data acquisition occurring at new facilities within the UPCI. As the newly developed fluidics technology becomes integrated and optimized at the UPCI facility using cell lines capable of osteoblastic differentiation, we will begin analyzing primary donor osteoblastic cells from the HMS collaborator for optimal conditions for growth and for age-correlated differences in differentiation potential.

Issues to be faced include determination of best approaches for incorporating multiple needles and/or multiple channels within each needle for efficient fluidics routines to be performed across multiple wells in 384 well plates, and whether such devices should be integrated within the system and coordinated with monitoring or whether plates should be removed and replaced in the imaging system using off-line fluidics devices.

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute (NHLBI)

PROJECT TITLE: Histo-Mechanics & Biology of Remodeling in Hypertension

ABSTRACT:
Hypertension remains a major risk factor for many cardiovascular diseases, and thereby is responsible for significant morbidity and mortality. Recent advances in vascular biology and mechanics suggest a paradigm shift in hypertension research. It is now clear that focusing on local regulatory activities of the vascular wall that are controlled by mechanotransduction mechanisms promises significantly increased understanding. In this proposal, we will focus on the molecular mechanisms of vascular adaptation in coronary and cerebral arteries and arterioles, and the associated integrated manifestations in vessel morphology and function at the cellular and tissue levels. Toward this end, we have developed a new micro-pig model of renovascular hypertension that allows us to detail the time-course of hemodynamic changes during the development and reversal of the hypertension. Using an externally controllable suprarenal aortic coarctation model, we will delineate between purely mechanical effects and those due to engaging the renin-angiotensin system. This will allow us to explore the hypothesis that the efficacy of pharmacological therapy depends strongly on the target vascular bed and the time that the intervention is initiated during the development of the hypertension. The overall working hypothesis is that hypertension-induced alterations in cell function and matrix biology are largely due to changes in the point-wise multiaxial stress field. Specifically, we hypothesize that altered stresses (intramural and wall shear) induce (1) changes in the local expression of nitric oxide and angiotensin, (2) down-regulation of potassium-sensitive ATP channels and adenosine receptor subtypes, (3) increases in RGD integrin binding sites in the matrix, similar to those in a wound healing response, and (4) spatial and temporal differences in apoptosis and the production of growth factors and proteases. These effects, balanced by a resetting of the baroreceptor reflex, shear stress regulation of endothelial activity, and the myogenic response together result in the bed-specific adaptation. These hypotheses will be tested by combining clinical, molecular, cell biological, immunohistochemical, morphological, and biomechanical methods to study coronary and cerebral vessels (n = 5-8 per cohort) at multiple times during the development and reversal of hypertension in a single animal model – although there are many calls in the literature for multidisciplinary attacks on the problem of hypertension, this study will be the first to collect and synthesize such broad data. Indeed, given the vast amount of data, we suggest that combining three recent, separate theoretical developments by members of our team will enable us to develop

mathematical models that synthesize the data and provide predictive capability. The latter will enable the exploration of further hypotheses in an efficient manner and guide pharmacologic delivery strategies. Years 1-2 will focus on the time-course of changes due to the development of hypertension whereas years 3-5 will focus on the time-course of changes due to reversing the hypertension either mechanically or via specific pharmacological agents, both as a function of the time (during the development of hypertension) that the intervention is initiated.

STATUS OF RESEARCH AND PARTNERSHIP:
There have been no changes in the partners or the organizational structure. As this project has developed, three interconnected phases have revealed themselves. First, the development of a new animal model, theoretical framework, antibodies, and a coordinated effort to collect a diverse set of data by multiple labs using tissue from the same animals. Second, the collection of sufficient sets of data to demarcate the time-course of hypertension induced changes in cell / tissue structure and function. Third, the synthesis of data into a unified theory. We have made tremendous progress with regard to the first phase and are now well into the second phase. In particular, over the last 9 months, 6 papers have appeared or are in press. These include a paper on our new animal model, one on a possible mechanism of collateralization near the coarctation, two on the theoretical framework, one based on a computational model, and one review article. Two other papers are in review and others in preparation. These papers represent the requisite foundation for many aspects of our work, and we are now well into the important second phase wherein large data sets are being gathered at multiple end-points during the development of the hypertension.

ISSUES:
Our primary issue in the beginning months of this partnership was the recruiting and training of new graduate students, residents, post-docs, and technicians for multiple laboratories and then coordinating their harvesting and testing of tissue from the same experimental animals. This issue was resolved fully during the summer of 2002, and since that time the team has worked together remarkably well not only on planned experimental days but also when faced with last minute changes in schedule due to emergency procedures on the animals. As we noted at our last group meeting (attended by 16 of the 18 members, including students and faculty), the coordination of personnel across the 6 departments has become almost transparent – we have become a true team.

The only ‘issue’ that has arisen, which is not a function of the partnership, is an unexpected difficulty in securing sufficient animals (a particular micro-pig) this summer. This requisition problem is introducing some delays, although we shall use the extra time to focus on theoretical aspects as well as data analysis and coordination.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Allergy and Infectious Diseases (NIAID)

PROJECT TITLE: Drug Discovery of Large-Scale Variant Targets by HTS

ABSTRACT:
This project involves a collaboration between Novasite Pharmaceuticals, Inc. and the University of New Mexico (UNM). It involves development of a novel flow cytometry-based (FCM) high-throughput screening (HTS) system capable of screening and sorting thousands of cells bearing variant receptor targets simultaneously in real time. We will use this instrumentation to develop a novel approach to drug discovery via large scale generation and screening of variant targets, centered on identifying ligand-receptor interactions at large scale. The UNM team is developing the FCM hardware, while Novasite is utilizing proprietary technology to develop a library of receptor variants with a single base substitution per variant and a cell system where a single variant receptor type is expressed in a single cell. The combined advantage of the HTS FCM and variant target expression technology is a combinatorial explosion in the number of ligand-receptor interactions explored relative to one-receptor screening approaches. Our aim is an agonist for the cannabinoid-2 G protein coupled receptor (GPCR), which may prove useful as an anti-inflammatory and immunosuppressive agent. A general expression system efficiently tranfects one single variant target cDNA per cell in a single transfection step. We use a cell-based GPCR functional assay based on Ca2+ sensitive fluorescent dyes. The GPCR's binding site residues are randomized, resulting in thousands of receptor constructs with enhanced recognition properties, capable of recognizing novel and high affinity leads. For lead optimization, we will sort cells with lower EC50s than the wild type receptor. The variant GPCR present in these isolated cells represents a mutation that enhances the potency of a given lead. This data will be analyzed by computational molecular models, matching the variation of chemical moieties within the ligand with the variation of amino acid residues within the receptor to guide docking procedures. Translating the amino acid changes that enhance the lead's potency into mirror-image modifications proposed on the chemical compound will guide the lead optimization process.

STATUS OF RESEARCH AND PARTNERSHIP:
Hypercyt sorting by UNM completed according to original Research Plan. Novasite engineers developed System 4 so that analysis/sorting can be performed with increased fidelity. Software was developed to analyze data, determine response thresholds, calculate statistical parameters and present the results. We proved that 1) single cell screening is over 25-fold more sensitive than standard formats allowing resolution of subtle activity differences, 2) single cell technology

permits simultaneous analysis of at least 20 different variant receptor-bearing cell types in a population, 3) at least 15 different color-coded populations can run at once allowing simultaneous evaluation of at least 300 different variant receptors (20 variants/population x 15 populations), 4) a total hit rate of nearly 28% was observed using WT plus 200 GPCR variants validating on an industrial scale the utility of variants and single cell screening technology for finding novel hit compounds, 5) affinities of ligands for closely related receptors using standard pharmacological approaches can be multiplexed validating the utility of the system for performing high-throughput pharmacological analyses necessary for characterizing molecules active against different variant receptors. We have begun to characterize some of the 40 novel compounds prepared by Dr. John Huffman (Clemson University) against WT CB2 receptor and some variant CB2R bearing cells to develop selective CB2R ligands. The compounds follow a structure activity relationship developed by Dr. Huffman, Dr. Patricia Reggio (Kennessaw State University) and Dr. Ballesteros (Novasite) according to homology modeling and rational drug design principles. Results have been presented in several public conferences.

ISSUES:

Our greatest challenge this year has been the development of a cell-based assay system suitable for resolving CB2R specific activation from non-specific cell activation when studying lipid-like or hydrophobic ligands. It has been difficult to find host cells that are devoid of inherent responsiveness to cannabinoid ligands making it hard to resolve a CB2R specific response from an endogenous response. CB2R is representative of a relatively recently appreciated class of receptors (EDGR family, cannabinoid receptors, LPA receptors) that recognize lipid ligands and which dictate a unique approach in ligand design and assay system design. Recently we have made progress in defining a cell-based assay system suitable for generating a CB2R specific response. Once the assay system is fully developed, the rapid analysis capabilities of the hardware and software will enable us to very quickly characterize the compounds and the 200 variant CB2 receptors that have been prepared during the past year and will guide development of a selective lead molecule.

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung and Blood Institute (NHLBI)

PROJECT TITLE: Bioengineering design of artificial blood

ABSTRACT:
Our objective is the design and development of artificial oxygen carrying plasma expanders (OCPEs) based on the modification of the hemoglobin molecule aimed at formulating an oxygen carrying fluid that has comparatively high viscosity, high affinity for oxygen, high oncotic pressure and that is economic in the use of hemoglobin, i.e., is effective with a minimal concentration of hemoglobin. These goals are being achieved via surface attachment polyethylene glycol (PEG) to the hemoglobin (Hb) molecule. Variables in PEG attachment include length and number of PEGs, bifurcations and bending moments. On biophysical considerations each variant has different solution properties, that may affect oxygen binding. A PEG formulation has been optimized in terms of cost, biological efficacy, COP, vasoinactivity, vascular retention and viscosity. Physiological research in the microcirculation was performed for further understanding the foundation of tissue oxygenation and is used to explore how alterations of blood physical properties affect tissue oxygenation and tissue survival in extreme hemodilution and shock. This program emphasizes the comprehension of the mechanism necessary for a stable balance between NO scavenging by molecular Hb in solution and the production of EDRF by shears stress dependant mechanisms. Different OCPEs will have different effects in this process leading to different types of vasoactivity. The product is now finalized and was tested by Sangart, Inc., San Diego, in clinical trials phase 1 at the Karolinska Institute of Stockholm. The trial was successful and phase 2 trials are being designed.

STATUS OF RESEARCH AND PARTNERSHIP:
The focus of the initial activity has been the implementation of the research and development plan leading to the design of an effective product that can be manufactured and delivered at a cost that is competitive with blood. The problem of effectiveness was addressed by establishing a control baseline in terms of existing products. An array of microvascular tests were made at UCSD to determine the microvascular transport properties of an oxygen carrying bovine molecular hemoglobin solution manufactured by Biopure Inc. presently marketed for veterinary applications. Analysis of the effectiveness of this product was made in terms of functional properties of the microcirculation during extreme hemodilution and shock and comparing this with conventional non oxygen carrying plasma expanders (dextran 70kDa, hydroxyethyl starch). It was found that this type of molecular hemoglobin based product provides no functional improvement over that attainable with conventional colloidal plasma expanders, supporting the need for a radically new approach which was attained by with MaleamidePEG-Hb. For periods

of 2 hours following resuscitation, this product was found to be superior to conventional plasma expanders and blood in terms maintenance of functional capillary density, acid base balance, perfusion, and survival. This product was been submitted for evaluation in terms of toxicity, biodistribution, intravascular retention time and systemic cardiovascular effects in accordance to the requirements for application for clinical trials by Sangart Inc., of San Diego which were successful leading to the Development of production facilities and a Phase 1 clinical trial that were successful.

ISSUES:
None.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB)

PROJECT TITLE: Biomedical Optics for Medical Research and Clinical Care

ABSTRACT:
The goal is to establish a consortium of investigators on the medical school (Oregon Health & Science University-OHSU) and engineering center campuses in Oregon. Aim 1: Establish a Biomedical Optics Laboratory on the OHSU campus as a central resource supporting the interface of engineering centers with biomedical research and clinical studies. Aim 2: Initiate biomedical optics projects in the field of tissue engineering and biomaterials development. Aim 3: Initiate biomedical optics projects in the field of cancer detection, imaging and treatment, and cell biology. Aim 4: Develop the partnership between bioengineering and medical research by establishing a program identity which reinforces formal communication amongst participating investigators, staff, and students and represents a visible program to potential new collaboratores in the medical centers.

STATUS OF RESEARCH AND PARTNERSHIP:
Aim 1: Establish the Biomedical Optics Laboratory. The laboratory is established with space and resources within a cell and molecular biology laboratory in the Dept. of Dermatology. Engineering center collaborators include the Oregon Medical Laser Center at Providence St. Vincent Hospital and the Oregon Graduate Institute (Dept. of Biomedical Engineering) which has merged with OHSU as the School of Science and Engineering.

Aim 2: We have initiated several biomedical optics projects:

2.1. Novel confocal microscopy design for imaging green fluorescent protein expression in cartilage underlying a light-scattering skin layer in mice.

2.2 Polarized light imaging of skin cancer to guide Mohs surgery.

2.3 Optical fiber spectroscopy of skin and other tissues, introducing novel catheter designs and basic theory on how fiber collection efficiency is affected by tissue optical properties.

2.4 Photodynamic therapy of cutaneous sarcoids in horses, and other veterinary applications.

2.5 Optoacoustic imaging of vascular and melanotic skin lesions.

2.6 Optical coherence tomography and relationship of speckle to tissue optical properties.

2.7 Low-coherence interferometry to monitor vibrations of the cochlear membrane of the inner ear.

2.8 Optical measurements of strain using laser speckle in engineered tissues and applied to cells with optical tweezers exerting the driving force.

Aim 3: Partnership development. We are establishing collaborative relationships with local industrial partners with innovative laser technology. We have begun a collaborative research project with the local undergraduate school, Portland State University.

ISSUES:
The process of establishing the working relationships between three different institutions has been bumpy, especially with the merger of two of the institutions which confused the scene. But this third year has seen a smooth operation. Perserverance and patience are the best tools for fashioning such a consortium.

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GRANTING NIH INSTITUTE/CENTER: National Cancer Institute (NCI)

PROJECT TITLE: Integrative Biology of Tumor Angiogenesis, Invasion and Metastasis

ABSTRACT:
Now that numerous important genes associated with tumor angiogenesis, invasion and metastasis have been discovered, the grand challenge is to understand their function in intact animals. The second major challenge is to integrate and apply this knowledge to cancer prevention, detection and treatment. In our BRPG, we meet these challenges with a new, more precise, quantitative, integrative and multi-disciplinary bioengineering approach. This new bioengineering approach builds on unique and innovative techniques such as 1) genetically engineered mice to visualize gene expression, 2) in vivo models to visualize molecular and cellular events, 3) computer-assisted in vivo microscopy to quantify gene expression and function continuously and non-invasively at high (1-10 μm) resolution in intact animals, 4) mathematical modeling to integrate the resulting information. Using this powerful technology, we investigate four critical aspects of tumor metastasis: angiogenesis, invasion, hematogenous metastasis, and lymphangiogenesis & lymphatic metastasis. Key accomplishments during years 1-3 are: i) deeper quantitative insight into expression and function of three genes (NO synthase, VEGF-A, VEGF-C) considered critical to these four aspects of metastasis; ii) established a quantitative link between cell traction force and invasion through the tissue matrix; iii) critically tested the long-standing but unproven hypothesis that angiogenesis facilitates metastasis by increasing cell shedding; iv) demonstrated that functional lymphatics are not present inside human tumors but lymphatic metastasis occurs from functional lymphatics in the tumor margin. Years four and five will see integration of these data in a unified framework and identification of strategies for clinical translation. The proposed BRPG offers a new paradigm for integrative studies of the dynamics of gene expression and function in cancer. With this new paradigm available to our collaborating partners working at the forefront of genomics and proteomics, this BRPG will facilitate translation of knowledge about the molecular biology of cancer into effective cancer prevention, detection and treatment strategies.

STATUS OF RESEARCH AND PARTNERSHIP:
Project 1: Vascular Angiogenesis: In collaboration with Dr. Donald G. Buerk, We found that nitric oxide (NO) mediates angiogenesis in solid tumors and highly metastatic variant tumors produce more NO and exhibit more but smaller tumor vessels. Chronic NO inhibition resulted in larger and less tortuous vessels. In collaboration with Dr. Paul L. Huang, we discovered that eNOS but not iNOS in host stromal cells contributes to angiogenesis and vessel morphogenesis in tumors. Although chronic NO inhibition lowers tumor tissue oxygen levels and slows initial tumor growth, response to vasoactive agents is increased suggesting improved tumor blood flow manipulability. We also uncovered paracrine regulation of angiogenesis and adipogenesis through VEGFR2 signaling paving the way for understanding the link between obesity and cancer.

Project 2: Invasion: By using cellular and molecular reagents developed by Drs. Michael Klagsbrun and Bruce Zetter, we have shown that VEGF plays a dose-dependant role in cancer cell mobility. The degradation of collagen type I telopeptides (non-helical domain that participates in collagen polymerization) enhances tumor cell invasion. The enhanced invasion was related to a reduction in collagen rigidity.
Project 3: Hematogenous Metastasis: By using cellular and molecular reagents developed by Drs. Josh Fidler and Brian Seed, we have established an orthotopic model of renal cell carcinoma in mice that allows us to measure the rate of cell shedding by a renal tumor. The transcriptional and functional analyses revealed that CD44, a3 integrin and caveolin were downregulated in the shed tumor cells and shedding is a passive process facilitated by reduction in cell attachment through a3 integrin or CD44.
Project 4: Lymphangiogenesis and Lymphatic Metastasis: In collaboration with Dr. Kari Alitalo, we discovered that intratumor lymphatics do not function despite the presence of the lymphangiogenic molecule VEGF-C and its receptors VEGFR2 and R3 in tumors (Leu et al, Cancer Research, 2000). Furthermore, in collaboration with Dr. Peter Carmeliet, we showed that VEGF-C increases angiogenesis and growth in tumors without altering leukocyte-endothelial interactions (Kadambi et al, Cancer Research, 2001). In collaboration with Dr. David Jackson (Oxford) and Dr. Stanislav Tomarev (NIH), we showed that the recently discovered lymphatic marker (LYVE-1) is also present in the sinusoidal blood vessels of the liver, but absent in the primary and secondary liver tumors (Carreira et al., Cancer Research, 2001). We then used two-photon microscopy to image deeper functional lymphatics (Padera et al., Molecular Imaging, 2002) and found them to be absent in both animal and human tumors (Padera et al., Science, 2002). We also demonstrated that these tumors still metastasize to lymph nodes, in spite of the lack of functional intratumoral lymphatics, suggesting that lymphatics in the tumor margin are the pathway for lymphatic metastasis (Padera et al., Science, 2002). Finally, we have shown that compressive mechanical forces generated by proliferating tumor cells can collapse both blood and lymphatic vessels and render them non-functional.

ISSUES:
The Bioengineering Research Partnership is an ideal and innovative program to integrate bioengineering with moleculart biology and molecular medicine, and to facilitate translation of new knowledge from genomics and proteomics to improving health care and quality of life.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB)

PROJECT TITLE: Plant viruses as platforms for biomaterials

ABSTRACT:
The overall aims of this project are to explore virus-based protein cage structures as platforms for synthetic modification with direct applications in bioimaging, and targeted drug delivery. The investigators involved in this project have significant experience working in the area of structure –function relationships in viral and related capsid systems. The virus capsid proteins are highly symmetrical supramolecular assemblies and these structures have a number of distinct advantages for their use as precursors for nanomaterials. A) They can be produced with relative ease in large amounts using either their native hosts (plants) or heterologous expression systems (E. coli, P. pastoris, baculovirus). B) An in vitro assembly system has been developed, which allows for disassembly and reassembly of capsid proteins. C) A wide range of genetic mutations can be accommodated by the viral capsids. D) Synthetic methods have been developed for chemically modifying the viral capsids using either endogenous or engineered functional groups. E) Methods and expertise for structure determination are in place to evaluate the structure of modified capsids.

STATUS OF RESEARCH AND PARTNERSHIP:
Work has been initiated between members of this partnership and significant progress has been made towards achieving the goals of the project. In particular the following interactions have been initiated: Johnson-Doerschuk image reconstruction; Johnson-Finn development of CPMV constructs and attachment of organics to CPMV; Young-Douglas development of CCMV constructs and attachment of organic and inorganics to CCMV; Johnson-Young-Douglas image reconstruction of CCMV constructs; Johnson-Young-Zlotnick development of CCMV assembly system.

Members of the partnership have participated in two focused meetings as opportunities for presenting current and future research. One of these meetings was held in Big Sky Montana (September 2002) and included members of the partnership (P.I.s) and selected individuals with a significant interest in the use of protein cages as templates for nano-materials (NASA-Ames, UC Berkely, The Scripps Research Institute, Montana State University). A total of 25 investigators were in attendance. The second meeting was held in La Jolla at The Scripps Research Institute and included all P.I.s from the partnership, post-docs, graduate students, and undergraduates. Additional investigators, outside the partnership, with an interest in the broad goals of the

research were invited and encouraged to attend (Rice Univ, UCLA, Caltech, Montana State, The Scripps Research Institute). There were 85 people in attendance at this meeting.

ISSUES:
As evidenced by the activities of researchers beyond the partnership, and their interest in participating in the meeting the partnership has organized, it is clear that there is a growing interest in the use of viral capsids (and related protein cage structures) as templates for a new generation of nano-materials. How can the the partnership respond to this interest, through additional collaborations/interactions without losing the focus and synergy among members, which is a very strong aspect of this partnership?

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GRANTING NIH INSTITUTE/CENTER: National Cancer Institute (NCI) and National Institute of Biomedical Imaging and Bioengineering (NIBIB)

PROJECT TITLE: DIGITAL MAMMOGRAPHY WITH A HIGH RESOLUTION FLAT PANEL IMAGER

ABSTRACT:
This bioengineering research partnership with Fairchild Imaging, Inc., (formerly, Lockheed Martin Fairchild Systems) is aimed at developing and evaluating a new high-resolution flat-panel mammographic imager with variable pixel size (40 and 80-microns). This next generation imager is a 2 x 3 array of large-area CCDs (8 cm x 8 cm) tiled in a seamless fashion to provide an imaging area of 16 cm x 24 cm. The CCD array is coupled to a structured CsI:Tl scintillator using non-tapering (1:1), straight fiberoptics, thereby preserving the spatial resolution without the detrimental loss in the collected signal, which is common with the older generation that use tapered fiberoptics. Our experience with the 100-microns pixel GE clinical evaluation prototype in a screening population appears to demonstrate equivalency for cancer detection with similar sensitivities. However, there are concerns about the ability to detect more subtle forms of calcifications such as punctate and amorphous. When calcifications are visualized the edges do not appear to be as sharp as that observed with spot film views, which may be related to the relatively large pixel size (100-microns) of the detector. Hence, this investigation was undertaken with the specific hypotheses stated in the application. The specific aims of the research have not been modified. The research plan calls for preliminary computational and experimental studies followed by development and comprehensive evaluation of the system through objective and universally accepted metrics such as the spatial frequency dependent modulation transfer function and the detective quantum efficiency.

The program began on July 1, 2001. In last year, the research activities stated in the following section have been accomplished.

STATUS OF RESEARCH AND PARTNERSHIP:
In last year, the following research activities have been accomplished:

1. Model refinement: A cascaded linear systems based model was developed in the first seven months of the grant to theoretically investigate the potential imaging characteristics of the proposed system. This model has been refined to accurately estimate the electronic noise in the imager. The refined model reiterates the results of our preliminary computations and provides support for the stated specific hypotheses.

2. Development of a single module prototype: A single module 8x8-cm prototype, which operates at a pixel pitch of 39-microns has been developed and experimental determination of the electrical and imaging parameters are in progress.

3. Scintillator evaluation: In the first seven months of the grant, structured CsI:Tl scintillators from two possible vendors varying in thickness and manufacturing processes such as substrate, coating type and strength, were evaluated using a laboratory 1 inch x 1 inch back-illuminated CCD operating in the 24-microns pixel mode in terms of signal intensity, sensitivity, spatial resolution properties in terms of the modulation transfer function (MTF), and DQE. This resulted in identification of the appropriate thickness range for the CsI:Tl scintillator. CsI:Tl scintillators within this thickness range are currently being characterized with the single module 8x8-cm prototype. Further we are also investigating a new pixilated structured CsI:Tl scintillator developed by one manufacturer and preliminary results demonstrate excellent imaging properties.

4. Fiberoptic evaluation: The appropriate thickness of fiberoptic plate required to provide adequate protection to the CCD has been identified and incorporated into the single module prototype.

5. Electrical properties of a single module: The electrical noise properties of the single module 8x8-cm prototype were investigated at room temperature.

The experimental verification of the objective and universally accepted metrics such as the spatial frequency dependent modulation transfer function and the detective quantum efficiency with the first single module prototype, indicate that this new generation of digital mammography system would provide the highest spatial resolution (greater than 10 cycles/mm) and improved DQE (greater than 65% at zero-frequency) than the systems currently in clinical use.

ISSUES:
There are no outstanding issues.

One manuscript addressing the verification of the refined cascaded linear systems based model and applied to an amorphous silicon based imager was presented at SPIE 2003: Physics of Medical Imaging, February 2003 at San Diego, CA.

Two manuscripts, one based on the experimental data and the other based on the theoretical model and simulations are currently under preparation for submission to peer-reviewed journals.

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GRANTING NIH INSTITUTE/CENTER: National Institute on Aging (NIA)

PROJECT TITLE: Iron Metabolism Alterations in Alzheimer's Disease

ABSTRACT:
Our BRP applies minimally invasive technologies to determine if altered brain iron metabolism in the face of Mild Cognitive Impairment (MCI) represents a significant risk factor for the development of Alzheimer's disease (AD). Goals are development of biomarkers for the diagnosis of AD based on a novel MRI imaging technology and peripheral blood assays (lymphocyte flow cytometry, genomic analysis) evaluating perturbed brain iron metabolism. Sequential MRI and blood studies are correlated with periodic psychometric evaluations of 75 MCI subjects and 25 normal controls over a 4 to 5 year period. The MR imaging technology will be validated by application to mice with an engineered deletion of the iron regulatory protein 2 (IRP-2) gene. This animal accumulates excessive amount of brain iron and and develops a neurodegenerative disorder. Direct assays of brain iron in its various forms ("free," transferrin bound, ferritin, non-heme, total) will be correlated with MRI signals from specific voxels and results extrapolated to human imaging studies. Dr. Tracey Rouault (NICHD), a consultant to the project, has developed this animal model and enabled us to develop the colony.

Inclusion and exclusion criteria for MCI and control subject recruitment has been defined with our project consultant, Dr. Ronald Petersen (Mayo Clinic). After screening with an interview, medical history, Mini-Mental score (Folstein) and Wechsler logical memory test, a consensus conference selects subjects for more detailed psychometric evaluation (Dr. Britt). After this study, subjects are assigned to the MCI, control group or excluded. 415 subjects have been screened over the past 9 months and 15 selected as MCI and 26 controls. The selection process conforms to NIH regulated age, gender, and racial/ethnic considerations. Evaluators have obtained certification from the Washington University Alzheimer's Study Group to make Clinical Dementia Ratings (CDR). (Sequential CDR's are videotaped). Once selected, subjects have a yearly MRI and biannual blood and psychometric evaluations. A 15% annual conversion rate of MCI to AD is anticipated. A statistical data base has been constructed (Access) and implemented for storage, retrieval, and analysis with our statistician Floyd Petersen MPH.

STATUS OF RESEARCH AND PARTNERSHIP:

Human Studies: Susceptibility weighted imaging sequences and spectroscopy have been acquired on normal volunteers using variations of TR/TE/ and flip angle to optimize parameters to cover appropriate anatomical areas and yield optimal gray - white matter and CSF contrast. MRI data is analyzed for segmentation, volume calculations and presumed iron accumulation. Proton MR spectroscopy using a single voxel short echo time technique in the area of the cingulate gyri and short echo time PRESS technique through the bilateral hippocampi has been implemented. Sequences and parameters are stored as an imaging protocol. 15 cases have had detailed psychometric evaluations, MR imaging, spectroscopy and blood tests to include genomic assays. Our 6 month psychometric and blood evaluations will be in place in June 2003 for cases selected in November, December 2002.

Mice Studies: The 11.7 Bruker software interface has been tested on normal mice and FeCl2 phantoms. The "IRP-2 knockout" mouse colony has been initiated and animals for imaging and biochemical assay will be available within 4 months – fall 2003.

Biochemical: "Free" and protein bound brain iron assays, flow cytometry, DNA and plasmid extraction, immuno-cytochemistry, and isolation of human lymphocytes from whole blood have been implemented. A microfluorimetric assay for brain "loosely bound iron" has been established. Human blood samples have been received and processed for quantification of IRP-2, amyloid precursor protein (APP), ferritin, ubiquitin, transferrin, transferrin receptor, and genomic analysis. Iron and other divalent cation assays are conducted by both microfluorometry and atomic absorption spectroscopy.

ISSUES:
Subject selection to meet MCI criteria is stringent. An on-site review by our consultant Dr. Ronald Petersen (Mayo Clinic) has facilitated our selection process. All cases selected up to the time of Dr. Petersen's visit (41 cases, 15 MCI, 26 control) were reviewed with attention to the statistical demands presented by the relatively small N and uncertainty inherent in our ultimate final diagnosis. In view of attrition in the elderly as well as diagnostic ambiguity we will increase our N (125 MCI, 51 control).

Initial imaging of normal mouse brain presented problems with full phantom and animal studies specific to the high field MRI (11.7T). Software changes and revision of the SWI sequences for small animals solved these problems. Genomic studies on fixed tissue and blood were compromised by formalin degradation of DNA. We have subsequently switched to analysis of snap frozen specimens with successful sequencing. Genomic studies focus on exon 5 of the IRP-2 gene that codes for the critical iron sensing nonopeptide sequence of IRP-2. Our attempts to produce a monoclonal antibody to the nonopeptide, iron degradation domain of IRP-2 have been unsuccessful because of the high degrees of domain conservation in normal mice. IRP-2 "knockout" mice do raise a significant immune response to the critical iron sensing nonopeptide sequence and this avenue is being actively pursued to create a monoclonal antibody for flow cytometric application. Our NIH funding commenced on October 1, 2002. This report covers 8 months of work.

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GRANTING NIH INSTITUTE/CENTER: National Center for Research Resources (NCRR)

PROJECT TITLE: Laser cell processing for basic and clinical research

ABSTRACT:
Numerous methods have been developed that rely on lasers to study and manipulate cells and tissues. Examples include inactivation of specific proteins or genes (e.g., chromophore-assisted laser inactivation), optoinjection of genes or macromolecules, activation of photosensitive agents (e.g., uncaging), photo-bleaching (e.g., motility/diffusion studies), and killing (e.g., cell purification). However, these potentially powerful techniques have been developed on low-throughput manual microscope systems, hampering their widespread use. To fully reap the benefits of these techniques, a novel automated imaging and laser-based processing technology for the analysis and manipulation of individual cells in a high-throughput manner has been developed. The technology, called the Laser-Enabled Analysis and Processing (LEAP) platform, has a number of unique attributes. The LEAP platform incorporates a moveable stage for plate handling, LED and xenon lamp illumination and excitation, adjustable magnification (2.5X - 20X), dual CCD cameras for imaging in brightfield, darkfield, phase contrast, or multi-color fluorescence, a pulsed laser with galvanometer steering for rapidly manipulating individual cells, and software for image/data analysis and process control. The LEAP platform rapidly captures images of cells in situ (e.g., in a well plate) through a combination of galvanometer and stage motions, thereby limiting stage movement (and cell displacement), while still achieving throughputs over 100,000 cells/sec. This speed enables reading of cell-based assays (e.g., live/dead, apoptosis, etc.) in 1536 well plates in <5 minutes. If desired, the laser can be fired at individual cells with specified criteria to achieve various cellular manipulations. LEAP has many potential uses, and this proposal brings together several institutions and researchers to develop and investigate the possible applications of this novel technology. For example, LEAP has been used in experiments to address the controversy surrounding the phenotype of the hematopoietic stem cell. In this case, LEAP was used to detect and eliminate the 0.01 – 0.30% of CD34+ cells that were left behind after two-pass flow cytometry sorting for CD34- cells, yielding a more accurate putative stem cell population that was used for in vivo stem cell assays. The more highly purified CD34- cells led to a different in vivo engraftment profile, indicating their more primitive status than CD34+ cells. LEAP has also been used for in situ purification and cloning of numerous adherent and non-adherent cell types. Further, LEAP was used to develop and implement RNA interference (RNAi)-mediated gene silencing (via optoinjection) in several cell-based functional genomics applications. Both direct RNA-mediated and indirect DNA-mediated (coding for siRNA) RNAi have been demonstrated, with results indicating that optoinjection has advantages over other transfection techniques with respect to simplicity, high cell viability, speed,

and selectivity. This unique combination of capabilities makes LEAP a powerful new analysis and laser-based cell manipulation technology with many potential applications.

STATUS OF RESEARCH AND PARTNERSHIP:
The clinical instrument for the tumor purging application has been completed. This instrument has been used in pre-clinical validation studies as well as other non-clinically related studies of cell purification. Due to feedback from FDA and the oncology marketplace, a pilot trial in tumor cell purging will no longer be pursued. The dramatic increase in successful use of monoclonal antibody therapies in NHL treatment has significantly reduced the enthusiasm for hematopoietic stem cell transplantation and/or a new tumor cell purging device. As described in the original proposal, a more flexible version of the instrument platform (called LEAP) has been developed to allow various types of non-clinical research. This instrument has benefited from the tumor purging instrument design, but with some critical additions: multi-color excitation and detection, multi-wavelength laser processing, and a more flexible user interface to facilitate research applications. The LEAP research platform has now been used in a variety of important applications including cell purification, cell transfection, and high-throughput high-content cell-based drug screening, thereby demonstrating the broad potential of the LEAP platform.

ISSUES:
The increasingly successful use of several monoclonal antibody products in the treatment of NHL has significantly reduced the scientific and market interest in hematopoietic stem cell transplantation and tumor purging in this application. This feedback from FDA and physicians has led us to drop the tumor purging pilot clinical trial from our plans. We instead will focus more on the research applications of LEAP including cell purification and transfection. These research applications have been increasing in importance over the past several years due to increased use of cell-based assays in life sciences and drug discovery research.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Arthritis and Musculoskeletal Diseases (NIAMS)

PROJECT TITLE: Novel X-ray Technology for Degenerative Joint Disease

ABSTRACT:
The overall goal of this Bioengineering Research Partnership is to integrate biological sciences, physics and engineering in the development and testing of a novel X-ray technology, called Diffraction Enhanced Imaging (DEI) for the non-invasive imaging of early cartilage degeneration for the eventual diagnosis of musculoskeletal disease, particularly osteoarthritis (OA). The technology, so established, may be expected to aid in the development of drugs and treatment strategies for the prevention and treatment of these and other soft tissue diseases. Conventional radiography and its variations, such as CT, rely on different absorption patterns within the object to give an attenuation-based image. Since cartilage tissue is almost completely transparent to x-rays, minimal absorption contrast can be detected, and therefore cartilage cannot be visualized on a plain radiograph. DEI is a novel means of utilizing synchrotron radiation to permit visualization of soft tissues and has the potential to revolutionize x-ray radiography. Our preliminary data show that use of DEI has permitted, for the first time, clear and detailed visualization of cartilage by using x-ray refraction and scatter rejection (extinction) in addition to absorption. With the aid of x-ray optical devices, soft tissue can be visualized via DEI as images with high contrast and very high resolution. Heterogeneities in contrast within the DE images of cartilage give rise to the “refraction/extinction features” observed. We have used DEI as a tool to investigate the joint structures of animals thus providing optimism that it may be used as a non-invasive radiographic diagnostic tool for early detection of OA. Importantly, the total dose of radiation exposure in DEI is not greater than the standard radiation exposure in the clinical setting at the same photon energy. The engineers of our team have developed a Multiple-Image Radiography (MIR) program which is related to DEI, but is essentially “the next generation”. MIR produces an important new type of X-ray image, based on X-ray ultra-small-angle scattering, which depicts fine textural features of tissue that are smaller than a pixel (50um). MIR also correct inaccuracies that are inherent in DEI. MIR is sensitive to very small deflections of the X-ray beam and exhibits almost total scatter rejection. Thus, MIR images have significantly greater contrast than conventional radiographs, allowing clear visualization of soft tissues such as cartilage, tendons and ligaments. Although DEI and MIR are currently being carried out with a synchrotron X-ray source, the techniques are not intrinsically dependent upon a synchrotron and independent studies are being carried out to transfer the technology to a compact X-ray source.

STATUS OF RESEARCH AND PARTNERSHIP:
In this, the first year of BRP funding, we have made progress in the radiographic imaging of articular cartilage of both human and animal synovial joints. An additional surprising application has been in the radiographic imaging of soft tissues other than cartilage. The partnership focuses on the further development and optimization of DEI technology for soft tissue imaging. The physicists of the team have recently installed a detector of higher resolution and greater efficiency than was provided by the imaging plates that had been utilized up to this point. This will allow acquisition of more data in a shorter period of time and, most importantly, will allow a real-time feedback so that specimens being imaged can be immediately re-positioned without the delay of image plate processing. Along these lines, an automated system for specimen repositioning outside the synchrotron beamline hutch is being developed. This will further enhance imaging efficiency. Particularly because of the close proximity (15 minute drive) of our two major partnership groups (Rush and IIT) we have been able to meet frequently to discuss results and to plan for future experiments. The partnership has been quite successful as attested to by our published abstracts and manuscripts.

ISSUES:
The only issue that has arisen has been the state of operation of the synchrotron beamline at Brookhaven National Laboratory in Upton, New York. Unfortunately, due to the age of the system, it is frequently in disrepair, often at times during which our group has scheduled beamtime. This reduces our data collection somewhat.

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Henry Ford Hospital (HFH): Department of Neurology

Technical University Graz, Austria: Institute of Biomedical Engineering

GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB)

PROJECT TITLE: Direct Brain Interface Based on Event Detection in ECoG

ABSTRACT:
A number of people with physical disabilities have difficulty performing any physical movement and would benefit from a direct brain interface (DBI), an interface that accepts commands directly from the brain. Our BRP partnership addresses the development and evaluation of a DBI based on detection of events in Electrocorticogram (ECoG) and the improvement of ECoG detection methods used to recognize specific brain activity.

Previous work with our DBI system has employed a detection method based on time-domain, template-matching methods for detection of event-related potentials (ERPs) in ECoG and demonstrated good accuracy in off-line experiments. Current efforts are focused along two inter-related directions: One aspect of our work is on-line implementation and testing of an ECoG based DBI with subjects at the UM and HFH who have implanted electrodes for purposes related to epilepsy surgery. (While these subjects are not members of the target user population, the presence of implanted cortical electrodes in these subjects provides a unique opportunity for DBI development). The proposed functional evaluation includes: 1) Development of an on-line, real-time testing system for ECoG based DBI methods; 2) Examination of the ability of subjects to learn to voluntarily improve the quality of activity-related brain events and hence detection performance given appropriate feedback; 3) Determination of the accuracy and speed with which a DBI can be used to perform functional tasks; and 4) Identification of the relationship between the location of electrocorticogram (ECoG) recorded brain events and the activated portion of the brain as observed through functional magnetic resonance imaging (fMRI).

The second aspect of this work focuses on improvement in detection methods. Previous work exclusively utilized a cross-correlation template matching method (CCTM) for the detection of event related potentials in ECoG. Improvements in the accuracy by which brain events can be detected are being approached through application of both time-domain/template matching and frequency-domain detection methods. The frequency domain methods are focused on detection

of event-related desynchronization and synchronization (ERD/ERS) which can occur with real or imagined motor activity. As these methods are tested and validated in off-line evaluation they will be implemented in real time feedback experiments outlined above. Accuracy achieved will also be compared to results from EEG based DBI systems. Detection methods are further being developed and evaluated to investigate their ability to differentiate between brain activity related to different actions.

The goal of this research is to demonstrate and evaluate the current and potential effectiveness of a DBI for control of functional tasks based on the detection of ECoG events recorded from subdural electrodes on the surface of the brain. It is intended that DBI methods developed through this effort will form the foundation for continuing future improvements in DBIs resulting in increased bandwidth and effectiveness. Beyond the scope of the proposed work, the results of these studies will form the foundation for clinical testing of the DBI with individuals from target user populations using subdural electrodes.

STATUS OF RESEARCH AND PARTNERSHIP:

On-line DBI Testing (at the UM and HFH):
The on-line DBI test system has been used with 8 subjects. While some subjects have demonstrated the ability to improve detection accuracy through this feedback system, other subjects have not shown improvements. An updated version of this system is planned that will shorten the delay between the subject's intent to activate the DBI and the presentation of feedback, which is viewed as a major barrier for subject improvement with feedback.

Correlation of fMRI and ECoG Detection Locations (at UM):
Data from fMRIs on normal subjects is being analyzed and final protocol adjustments are underway with actual experiments expected to start by fall.

Improved Detection Accuracy (at UM and Graz):
Current detection methods being investigated include a quadratic method based on the Neyman Pearson Test, adaptive auto-regressive parameters, and wavelets optimized with genetic algorithms. These new methods have the potential to combine detection of both time (ERP) and frequency-based ECoG events (ERD/ERS). The wavelet method in particular has shown some dramatic improvements over the CCTM method. As detection methods focused exclusively on detection of ERPs vs. ERD/ERS do not necessarily have the most accurate detection on the same channels, there is strong indication for a level of independence between these phenomena that warrant optimism for improved detection accuracy through combined detection.

ISSUES:

All collaborations are working very well. The success of the collaboration with the Technical University of Graz has opened new research possibilities and has actually become an issue because the original grant was written to fund this partnership for only the first three years of the grant. We are therefore pursuing an administrative supplement to additionally fund this subcontract for the remainder of the grant.

The official start date for this grant was 4/1/01. However, due to issues related to equipment ordering and student recruitment, we effectively moved our start date forward to 9/1/01 (through annual budget carry-over) and plan on continuing with this annual time frame for the duration of the grant.

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GRANTING NIH INSTITUTE/CENTER: National Institute for Biomedical Imaging and Bioengineering (NIBIB); formerly: National Heart, Lung, and Blood Institute (NHLBI)

PROJECT TITLE: Biomechanics of Leukocyte Adhesion Molecules

ABSTRACT:
This BRP was initiated to conduct interdisciplinary bioengineering research in the area of molecular biomechanics. Leukocyte and endothelial adhesion molecules govern the trafficking of cells in inflammation, immunity, cancer metastasis and other processes. Some adhesion molecules, among them the selectins, are specialized to mediate adhesion in the presence of blood flow. Pressure-driven blood flow is associated with a shear stress exerted on the vessel wall, which results in a force on leukocytes and other cells trying to adhere to the endothelium. It is believed that adhesion under shear stress requires adhesion molecules with rapid association rates (on-rates), resulting in rapid formation of bonds. In vitro experiments and modeling studies indicate that the selectins also have high rates of bond dissociation (off-rates). Preliminary data suggest that the off-rates of selectins vary systematically with the shearing force exerted on the cell bound by the selectin (reactive compliance or tensile strength). In addition, the release of at least one of the selectins is accelerated by proteolytic cleavage by a surface-bound or membrane integral metalloproteinase. The BRP has four specific aims. (1) To measure the bond lifetimes and apparent off-rates of L-, P- and E-selectin bound to their natural ligands. (2) To determine the role of L-selectin shedding in regulating leukocyte adhesion and selectin kinetics. (3) To determine the impact of the selectin length and their cytoplasmic tail for the biomechanics of adhesion under shear flow. (4) To design and build beads, liposomes and gas-filled bubbles (ultrasound contrast agents) that use leukocyte adhesion molecules to bind to vessel walls under shear stress. Each of these aims is approached in a three-pronged fashion. We propose to use laser trapping technology to directly measure biomechanical and kinetic parameters of selectin bonds, use single cells on sparse substrates to understand the biomechanics of selectins in an in vitro flow chamber, and use intravital microscopy to study selectin biomechanics in the context of the living organism. We use molecular biology techniques to manipulate cDNA, cells, and mice to isolate each molecular mechanism. The insights gained from basic science-oriented studies are used to design liposome-based targeted drug delivery systems and ultrasound contrast microbubbles for delivery in the vascular system under shear flow.

STATUS OF RESEARCH AND PARTNERSHIP:
The partnership is in active progress. We exchange reagents (antibodies, transfected cell lines) and technical expertise. The application of adhesion molecules for targeting purposes has been

particularly successful. Dr. Lindner has successfully imaged metastatic tumors, atherosclerotic plaques, inflammation and angiogenesis by targeted ultrasound contrast in mice. We have organized three Colloquia on the Biomechanics of Adhesion Molecules in 2000, 2001 and 2003. They were attended by approximately 50 scientists and graduate students each. This year, Dr. Lindner will be organizing the Fourth Colloquium on the Biomechanics of Adhesion Molecules with a focus on targeted imaging contrast and drug delivery.
We have recently discovered a way to greatly enhance targeting of microbubbles for molecular imaging. This was based on discoveries in Dr. Lawrence's and Ley's labs. Other areas are also progressing very well, with a very exciting paper coming out of the laser trap work which allows detailed comparisons of the adhesion strength of single E- and P-selectin molecules under controlled rates of loading.

ISSUES:
Producing sufficient amounts of one of the molecules, PSGL-1, for in vitro studies continues to be a challenge. Recently, we have obtained recombinant soluble PSGL-1 from two sources (glycosulfopeptides and a truncated Fc fusion protein). This has (largely) solved the problem. Nevertheless, we plan to build a recombinant protein production and purification facility during the next funding cycle to optimize availability and quality of ligands.

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PROJECT TITLE: Type I Collagen-Based Nerve Guide for PNS Regeneration

ABSTRACT:
The goal of this project is to design, engineer and evaluate in vivo a type I collagen-based nerve guide for peripheral nerve regeneration applications. The objectives of this project entail the isolation of the pertinent design parameters necessary for the initial prototype screening in a rat sciatic nerve model. Utilizing the optimal conditions from these results, a final prototype will be designed and evaluated in primate nerve models as a potential entubulation repair method for clinical applications. This second year report covers work conducted from June of 2002 through May of 2003. The key design parameters that have been investigated include permeability of the nerve guide membrane, axonal growth guiding channels (micro-tubes and filaments), cell growth inductive and cell adhesive properties.

The effect of permeability and guiding channels on nerve regeneration has entered the in vivo screening studies in rats. Repair of a 1 cm rat sciatic nerve gap has been completed using nerve guides having two distinctly different permeability properties; one prototype was permeable to small molecules such as sucrose (MW = 342 daltons) but not permeable to macromolecules the size of myoglobin (MW = 16,000 daltons), and the other prototype was permeable to carbonic anhydrase (MW = 29,000 daltons) but not permeable to catalase (MW = 270,000 daltons). All animals have been sacrificed after 6 and 12 weeks survival times. We are currently analyzing the results from these studies. Repair of a 1 cm rat sciatic nerve gap has been initiated using nerve guides containing one packing density each for the micro-tubes and the filaments. The nerve guides contain either five micro-tubes (each having an I.D. = 0.4 mm and wall thickness 0.025 mm in the dry state) or 32 filaments (each having a dry diameter about 90 micrometer). The nerve guide without the guiding channel served as a control and nerve autograft served as a standard. This study is ongoing.

We are evaluating the effect of gamma-sterilization and the storage conditions on the biological activity of cell growth inductive and cell adhesive molecules prior to evaluating these parameters in rat screening studies. The stability of bFGF-containing nerve guides stored at 22 C, 4 C and -20 C was initiated. The results suggest that the nerve guides containing bFGF molecules may have to be stored at -20 C or below. The stability of IGF-II and laminin molecules at various temperatures is being initiated. Another important factor to consider in the development of a bioactive nerve guide for clinical application is the effect of sterilization on the biological activity

of the molecules. Nerve guide membranes containing bFGF, IGF-II and laminin have been sterilized by gamma-irradiation (16 kGy and 25 kGy). These membranes are being evaluated in cell culture studies.

STATUS OF RESEARCH AND PARTNERSHIP:
In vitro cell culture and in vivo rat screening studies are being conducted at The University of South Florida (USF) under the direction of Dr. Frank Liuzzi.

Significant time and effort has been devoted to in vitro studies on the effect of bioactive molecules on cell (Schwann cells and PC-12 cells) growth, cell migration and neurite out growth. Several different staining techniques have been implemented to visualize cells grown on plain nerve guide membranes or nerve guide membranes containing bFGF, IGF-II or laminin. At this point the results are inconclusive due primarily to visualization difficulties. To overcome these staining difficulties, Propidium Iodide is currently being investigated to visualize cells grown on nerve guide membranes. We are also preparing thin nerve guide membranes (25 micrometer) with and without bFGF, IGF-II and laminin for cell culture studies as an alternative to the thicker nerve guide membranes (100 – 150 micrometer).

We have evaluated two of the four design parameters in a rat model at USF. The evaluation of the permeability parameter has been completed and the evaluation of micro-guidance channel parameter has been initiated. Cell inductive and adhesive parameters are being evaluated in vitro in preparation for in vivo screening studies in rats.

Primates are being trained for the efficacy studies to be conducted at Duke University under the direction of Dr. Roger Madison in anticipation of initiating the study in year three to year four.

ISSUES:
The primary issue has been monitoring and following up the progress of the partners. Recently, we have implemented a bi-monthly teleconference call meeting and have significantly improved the communication and productivity.

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GRANTING NIH INSTITUTE/CENTER: National Eye Institute (NEI)

PROJECT TITLE: Live microscopy and cytometry in vascular biology

ABSTRACT:
The broad aims of our program are to develop new technologies for visualizing, tracking, and quantifying cells in living animals, and to apply these technologies to important problems related to vascular biology in the eye. High-resolution cellular imaging in vivo will help gain physiologic insights beyond what can be obtained by traditional static methods such as histology and immunocytochemistry. Moreover, imaging individual live animals over time will substantially reduce the number of animals used as well as reducing statistic errors. We envision that the technologies developed in this BRP will have broad applications in vascular biology beyond the eye. The Specific Aims are:
I. Develop technology for cell identification, tracking, and quantification in vivo, with improvements in resolution, flexibility and speed of data acquisition, and methods of detection and quantification.

We are developing a flexible and transportable imaging platform with interchangable front ends so that the system can be easily converted from an in vivo confocal microscope to a scanning laser ophthalmoscope (SLO) for imaging different parts of the eye. This system will be upgraded in year two to enable high-speed confocal imaging at up to 200 frames/second. The high frame rate is needed for cell tracking experiments in the retinal vasculature, where the cells are moving too fast to track with the standard video frame rate of 30 frames/sec. This system will be used to study blood flow in animal models of diabetic and sickle cell retinopathy. In parallel, we are developing an SLO with wavefront sensing and correction technology to measure the optical aberrations of the eye, and to correct for the aberrations in order to achieve diffraction-limited resolution and to resolve individual cells in the retina. The technology development will be coupled with the development of fluorescent cell markers for imaging cell layers beyond the outer segment (photoreceptors), since we are particularly interested in visualizing the retinal pigment epithelium (RPE), which forms the outer blood-retinal barrier, and the choroidal circulation. Finally, we are developing an in vivo flow cytometer to detect and count individual fluorescently labeled cells in the circulation as they flow through a laser probe beam.
II. Apply live microscopy and cytometry to problems in vascular biology.

Dr. D'Amore will use the imaging system to study the vasculature in development and pathology in vivo. The role of specific growth factors (e.g. TGFb, VEGF) in vessel formation and stability will be determined (project II.1). Dr. Bogdanov and Dr. Hamblin will develop

molecular probes for vascular permeability and specific labels for vascular endothelial cells (EC) and RPE cells (project II.2). Dr. Bursell will investigate cellular mechanisms responsible for endothelial dysfunction in diabetic retinopathy. Microvascular changes in retinal hemodynamics and retinal leukostasis will be quantified in diabetic animal models using the high frame rate SLO (project II.3). Dr. Lutty will investigate vaso-occlusive processes in sickle cell retinopathy in vivo using transgenic mouse models of sickle cell disease, and determine which density gradient class of sickle RBCs initiates vaso-occlusions in rat retina (project II.4).

STATUS OF RESEARCH AND PARTNERSHIP:

In the first year of our grant, the technical team has been concentrating on developing in vivo imaging instrumentation for the mouse, while the biologic collaborators work on animal models that will be studied using the new imaging systems. We have developed an in vivo flow cytometer that detects bursts of fluorescence photons as individual cells flow through a probe laser beam in the vasculature of live, intact animal skin. We are working on software to automate cell counting. We have developed a line scanning confocal microscope (based on the bilateral scanning principle of Brackenhoff and Vischer) which can be adapted to an SLO. The scanning system is designed to achieve 200 frames/sec pending upgrade of the image acquisition hardware and software. We have also constructed a completely new SLO in Dr. Webb's laboratory with optics that have been designed specifically for the mouse eye. In addition the design has been implemented such that the necessary optical paths for wavefront sensing and for wavefront control are present in the SLO.

The wavefront sensing and correction modules are currently sitting on a separate platform in Dr. Burn's laboratory but will be integrated into the SLO in the near future. The Hartmann-Shack sensor uses a superluminescent diode (SLD) source, a lenslet array and 10 bit firewire camera, and is built to scale for a mouse eye. Software for the measurement of wavefront slopes is in place and working. We have not yet adapted our existing software for Zernike analysis, since we are concentrating on the wavefront control software first.

For the wavefront correction, we have established a strong collaboration with Dr. Tom Bifano's group, which is one of the premier groups developing micromachined membrane mirrors (MEMS Mirrors). These mirrors are now being developed commercially by Boston Micromachines Corporation, and they are also helping in implementing this program. This collaboration has allowed us to accelerate the project by bypassing the static phase plate correction technology as originally proposed for year 1, and move straight to the development of the active mirror control using a 144-channel Boston Micromachines deformable mirror. We have constructed a testbed for the measurement and control of aberrations, and are in the process of developing the hardware software systems for measuring wavefront slopes, and especially for changes in wavefront slopes.

ISSUES:

There are no major issues. There are some delays in filling positions and getting the program up and running quickly. Different teams in the Partnership are getting used to working together by participating in monthly BRP meetings and by working in one another's laboratories. On the technical side, the major deciding factor will be the tradeoff between making progress in programming the control software for wavefront sensing/correction, and the need to "freeze" some of the configuration for a while while making the mouse eye measurements. Also, the in vivo flow cytometer that just came on line is already being so heavily used for biologic measurements that we have to start building a second instrument for system optimization.

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GRANTING NIH INSTITUTE/CENTER: National Cancer Institute (NCI)

PROJECT TITLE: Multimodality biological Imaging of cancer / tumor hypoxia

ABSTRACT:
The main aim of this project is to deliver a comprehensive characterization of tumor hypoxia based on spatially correlated three-dimensional data sets derived from non-invasive imaging and tumor section analysis. Over the past year we have made significant progress in areas that constitute important components in the overall whole.
We have expanded our serial studies in tumor-bearing animals to include three promising PET imaging agents for tumor hypoxia. These are [18F] F-misonidazole (FMISO), [64Cu] Cu(II)-diacetyl-bis(N4-methylthiosemicarbazone) (Cu-ATSM) and [124I] iodoazomycin galactopyranoside (IAZGP). We have spatially correlated the intratumoral biodistributions of these agents in-vivo and related these to direct probe measurements of tumor pO2. PET imaging has been performed in two tumor cell lines grown in both mice and rats. In parallel to the in-vivo studies we have characterized the oxygen-dependent uptake and retention of these agents in cell lines in-vitro. We have discovered that the uptake of Cu-ATSM has a pronounced cell line-dependency and established a rank order of cell lines for Cu-ATSM uptake. These data were instrumental in the selection of suitable cell lines for in-vivo study. Preliminary work has also been performed correlating PET images with dynamic Gd-DTPA contrast MRI in the same animals.
We have developed our ability to perform microscopic analyses of tumor sections and have working assays for perfusion (Hoescht 33342 staining), cellular proliferation (BrdU) and hypoxia (pimonidazole). We can combine microscopic assays into composite images and examine the correlation between e.g., perfusion and hypoxia. In addition we are now developing our ability to map the endogenous indicators of hypoxia such as CA IX and HIF-1a. In conjunction with these fluorescent immunohistochemical assays, we have also been developing our abilities to perform digital autoradiography of radiolabeled molecules including [18F] fluorodeoxyglucose (FDG), [18F] FMISO and [125] IAZGP on tumor sections.
Central to the rational integration of these diverse data is the ability to generate a common three-dimensional coordinate system for all assays. We are now testing prototype fiduciary marker systems in animal models. The key feature of our current design is to enable the coordinate markers to remain non-invasive for PET and MRI but to be then converted to an invasive system for tumor sectional analysis.
In order to further investigate and validate the various hypoxia imaging agents we are developing tumor cell lines with hypoxia-sensitive reporter genes. We are focusing on cells expressing the

herpes simplex virus thymidine kinase/green fluorescent protein fusion (hsvtk-gfp) under the transcriptional control of hypoxia responsive elements. These cells will enable an alternative approach to non-invasive hypoxia imaging using the ability of the viral thymidine kinase to phosphorylate and trap [*I] 2'-fluoro-2'-deoxy-1-beta-D-arabinofuranosyl-5-iodouracil (FIAU). In addition the gfp component will enable the visualization of hypoxia on tumor sections by fluorescent microscopy. In this approach the mechanism of hypoxic selectivity will be HIF-1a stabilization rather than bioreduction of 2-nitroimidazole or Cu-ATSM. This model system will thus give us two independent assays of tumor hypoxia and should enable us to understand the relative impact of true hypoxia and restricted penetration of exogenous markers.

STATUS OF RESEARCH AND PARTNERSHIP:
This project formally started in October, 2001. The entire partnership meets regularly at approximately weekly intervals and as sub-groups more frequently than that. Now that the individual assays have developed or are developing sufficiently, the next phase of the work will entail the introduction of a common reference frame for all measurements. We anticipate this key step will require some trial and error to perfect but once accomplished will enable us to start integrating the diverse biological data into a coherent picture.

ISSUES:
None

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GRANTING NIH INSTITUTE/CENTER: National Cancer Institute (NCI) and National Heart, Lung, and Blood Institute (NHLBI)

PROJECT TITLE: Integrated Ultrasonic Systems for Non-invasive Therapy

ABSTRACT:
The ultimate objective of this 5-year Biomedical Research Partnership (BRP0 is to develop a unified body of scientific knowledge and validated technology concepts that are needed to establish ultrasound as a practical non-invasive treatment modality and to inaugurate ultrasonic therapeutics as a new biomedical discipline. We will systematically elucidate the spectrum of ultrasound therapeutic lesions that can modify various classes of diseased tissues, and we will develop integrated ultrasonic systems to position, induce, and monitor these lesions. We will focus on establishing a comprehensive basis for future treatments of cancer (primarily of the breast and prostate) and cardiac disease (primarily ventricular arrhythmia and myocardial insufficiency). These clinically significant diseases present challenging opportunities to test and refine our concepts, which have substantial implications for treating a broad array of problematic, life-threatening conditions.

This Biomedical Research Partnership involves biomedical engineering research at Riverside Research Institute; animal research studies at Weill Medical College of Cornell University (WMC) and Columbia University College of Physicians & Surgeons (CUCPS); and advanced systems development at Spectrasonics, Inc. Our multi-disciplinary research is designed to achieve a series of fundamental advances in the diverse areas involved in therapeutic ultrasound. We will employ extensive theoretical modeling to elucidate physical ultrasound-tissue interactions that can be used to produce therapeutic changes in diseased tissues. We will validate model results for thermal and mechanical effects in a series of animal experiments. Validated results will be used to design and implement advanced therapy systems incorporating ultrasonic arrays and real-time lesion monitoring. The systems will be tested and refined using animal experiments that investigate cancer and heart-disease therapy.

Our results will be incorporated in a systems model of ultrasonic therapy which will permit comprehensive treatment planning and design of future system features.

STATUS OF RESEARCH AND PARTNERSHIP:
Now in year 2, effective working relationships among partners have been established.

The focus is on controlled production of thermal ultrasound lesions to treat diseases through tissue coagulation. Riverside has refined 3-D computer simulations of high-intensity focused ultrasound lesion formation. Further modeling has explored key parameters in dual-

transducer monitoring of lesions by sensing differences in tissue stiffness with ultrasound radiation force.

Optical and electron microscopy have identified ultrasound lesion mechanisms: at lower temperatures structural proteins denature; at higher temperatures intracellular fluid vaporizes and lyses cells.

In-vitro experiments aimed at monitoring lesion formation have led to controlled tissue motion in the micron to millimeter range. Data acquisition timing is being addressed to improve discrimination between lesion and normal tissue.

Spectrasonics and Riverside designed a new system that integrates therapy and imaging ultrasonic arrays for therapeutic aiming, exposure, and monitoring. This system and associated diagnostic array were delivered and initial tests begun. An upgrade to capture digital radiofrequency ultrasound signals is currently being built. Associated therapy array transducers are being fabricated.

Columbia and Riverside worked on extensive in-vitro cardiac experiments; lesions were made that spare the epicardial and endocardial surfaces. The Riverside laboratory system that was delivered to Columbia last year was modified to form lesions in-vivo, synchronized to the beating heart. Open-chest dog experiments are due to commence this summer.

For cancer therapy, Cornell has measured ultrasound liver tissue properties and has made in-vivo lesions in rabbit livers. Lesion production in human tumor lines explanted in nude mice will commence soon.

ISSUES:
None.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and National Institute of Biomedical Imaging and Bioengineering (NIBIB)

PROJECT TITLE: BION Treatment of Neuromuscular Dysfunction

ABSTRACT:
In theory, a wide range of sensory and motor dysfunctions can be treated by electrical stimulation to evoke patterns of neural activity similar to those that underlie normal function. In practice, however, such stimulation has typically required relatively expensive and large devices implanted by a surgeon or skin surface stimulation applied by a trained therapist. We have developed a new class of generic devices that can deliver precisely metered stimulation pulses to an arbitrary number of nerve and muscle sites. BIONs (registered trademark; BIONic Neurons) are a new class of chronically implantable stimulators. They are single channel, wireless electronic microstimulators (16mm long x 2 mm in diameter) that can be injected in or near muscles and nerves. Each BION receives power and digital command data from a single, externally worn transmission coil to produce stimulation pulses with controlled current (0-30mA) and duration (4-512 microseconds). BIONs have been demonstrated to produce stable thresholds at their deployment sites and have been shown to be safe and effective for stimulating muscles in animals. Results from ongoing small cohort clinical trials have shown them to be effective in preventing and reversing shoulder subluxation and increasing knee function in patients with knee osteoarthritis. Under this BRP, we will design and build BION 1 implants and accessory components for testing, programming and controlling them in patients. We will develop and test a range of clinical applications to determine safety and efficacy and to further understand the mechanisms underlying neuromuscular pathology and treatment. In the first five years, these applications include activating and strengthening muscles in the shoulder, forearm and hand in patients suffering from stroke to reverse shoulder subluxation, minimize hand contractures and strengthen hand muscles to assist with constrain-induced therapy. Advances in BION technology, such as increased power efficiency, improved ASIC design and portability as well as sensor and back-telemetry capabilities for functional electrical stimulation (BION2S and BION2) will be deployed once their safety has been determined. In subsequent years, we will expand the clinical applications to provide more complete rehabilitation of multi-joint dysfunctions that commonly occur in stroke, explore other clinical applications and incorporate advanced BION2 technology to provide functional reanimation of paralyzed limbs using neural prosthetic control.

STATUS OF RESEARCH AND PARTNERSHIP:

All three clinical trials have received IRB approval from the participating institutions. Two of the three clinical trials, shoulder subluxation reversal and minimization of hand contractures in stroke patients, have either received, or been submitted to the FDA for IDE approval). Subjects are being recruited and evaluated for inclusion into these two trials. A preliminary clinical study is underway to develop and test EMG-triggered activation of BION stimulation, which will be used to control exercises to strengthen hand muscles to assist with constrain-induced therapy. In addition, pre-clinical feasibility studies to ascertain the use of BION technology to treat obstructive sleep apnea and to prevent pressure sores in spinal cord injury patients are currently underway.

Improvements in BION1 production and testing procedures have increased the yield of functional BIONs for clinical applications. BION1 production will be sufficient to meet the projected needs of the three NIH funded clinical trials and preclinical studies of other potential applications. Engineering test results of the BION2S ASIC, a direct replacement for the BION1 ASIC, indicate that power efficiency has been dramatically improved and that fundamental problems with the BION1 ASIC design have been resolved. Replacement of the BION1 with the BION2S ASIC does not require any significant changes to electronic subassembly and glass encapsulation manufacturing and is scheduled to take place within the next 8-12 months.

ISSUES:

1. The possible closure of Rancho Los Amigos National Rehabilitation Center by the Los Angeles County Board of Supervisors has prompted us to develop a back-up plan in case we have to move these two clinical trials to the University of Southern California.

2. Initial budgetary issues, more specifically calculation of the F&A cost associated with the grant and fund allocation to the three cores (manufacturing, regulatory and clinical), due to the fact that all three cores exist within one University were problematic and have been slow to resolve.

3. The clinical investigator in charge of the trial that was already underway with FDA IDE approval had to quit for health reasons, forcing us to reorganize personnel for that trial.

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GRANTING NIH INSTITUTE/CENTER: National Eye Institute (NEI)

PROJECT TITLE: Blind Pedestrian Access to Complex Intersections

ABSTRACT:
The pedestrian environment has become more challenging as transportation engineers have designed roadways to carry more traffic in less time. Wide arterial roads, actuated signalization, continuous flow designs such as slip lanes and roundabouts, and irregular intersection geometries are examples of intersection features that have made street crossings more challenging for pedestrians. This bioengineering research partnership of engineers, experimental psychologists, and rehabilitation professionals is working to improve access to complex intersections by pedestrians who are blind and visually impaired. The partners are engaged in research about basic perceptual and cognitive factors that affect orientation and mobility in complex pedestrian environments and the impact of various intersection design features on the safety and efficiency of pedestrians. These studies aid in understanding the nature of access problems that blind individuals experience. The team is also designing and evaluating new technologies for enhancing the orientation and mobility of persons who are blind as they negotiate complex intersections.
During Year 3, the WMU team continued to focus its effort on access to roundabout intersections and on engineering development work on the "Anti-veering training device", or AVTD. During this project year, the team submitted a paper detailing the findings of its research on auditory gap judgments at three roundabouts in the Baltimore/Annapolis area. We are nearing completion of a second paper reporting the results of a study in Tampa that evaluated how gap judgments are affected by variations in traffic volume. With the Vanderbilt team, we conducted a study comparing gap judgments with the actual crossing behaviors of persons who are blind and a pilot study of the use of underfoot tactile cues for non-visual alignment at complex intersections.
The AVTD project reached the end of the design and development stage in January, 2003. In January, 2003, a subcontractor began manufacturing 15 AVTD's. The target date for completing the first copy of the manufactured units is Summer, 2003. Final testing and redesign, if needed, is scheduled for August,2003. Manufacture of the balance of the units is scheduled for Fall, 2003. A paper detailing the engineering design of the device is in preparation.
Transportation engineers from the UNC Highway Safety Research group and North Carolina State University's Institute for Transportation Research and Education (ITRE) investigated the usefulness of VISSIM computer models to document the differences in the delays encountered by blind and sighted pedestrians at the exit lane at a single lane roundabout. The results suggest potential pedestrian treatments for improving the ability of blind pedestrians to safely cross

streets at roundabout intersections. These treatments will be evaluated "on-the-street" in the next phase of our roundabouts research. A paper developed from the modeling effort is under review.

STATUS OF RESEARCH AND PARTNERSHIP:
The Vanderbilt/Boston College teams completed a set of three experiments on audible pedestrian signals and submitted a paper for publication on the findings. The findings suggest two key changes in the design of audible street crossing signals. Vanderbilt's initial experiment on auditory motion perception also has been completed, showing that a "virtual reality" auditory display can yield an equivalent sound localization relative to actual sound sources. This work will now grow in two directions - an engineering focus on ways of making the computational algorithms more efficient and a psychoacoustics focus on using auditory motion displays to test the limits of spatial hearing with respect to alignment to moving sound sources

The Maryland School for the Blind team completed data collection for a study of eye gaze, in which the team evaluated where sighted and low vision subjects looked while crossing streets at complex intersections. The findings have been accepted for publication. This paper presents a detailed description of the methodology and the analysis techniques that allow the eye to be accurately placed on the scene. The Maryland team has also completed an extensive study on the issue of driver yielding behavior to pedestrians at roundabouts.

The Boston College team completed baseline data collection on blind pedestrians crossing at complex signalized intersections without APS in four cities. In year four, APS will be installed at each of these intersections, with post-testing planned to assess various aspects of street crossing safety and efficiency.

ISSUES:
Interplay between our research activities and public policy development in accessible transportation.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB)

PROJECT TITLE: Shape Memory Polymer Devices for Treating Stroke

ABSTRACT:
This project develops novel interventional devices for treating stroke. We are developing and testing two complementary devices: a mechanical clot extraction system and a neurovascular stent. The clot extraction system will address the current clinical need for an acute ischemic stroke treatment and the stent will address the chronic problem of stenosis and/or restenosis of the neurovasculature. Both of these devices utilize photomechanical micro-actuators based on laser-activated shape memory polymer (SMP). This multidisciplinary research is a unique combination of biomaterials, biophotonics, immunology/biocompatibility and clinical interventional neuroradiology.

SMP is a material that will have a significant impact on clinical medicine. SMP is a relatively new material that is similar to shape memory metals in its ability to actuate from an initial deformed shape into a second, pre-determined shape. Shape memory metals are currently very popular in medicine as a material for making vascular stents. SMP has advantages over shape memory metals for certain applications, including cost, higher recoverable strain levels, ease of manufacturing, better flexibility in navigating tortuous paths, and great versatility in fabricating extremely small, highly complex actuators. Potential applications of SMP include stents, stent release mechanisms, embolic coil release mechanisms, thrombus extraction devices, and many others.

The long-term goal of this research is to deliver clinical prototype devices that can begin FDA clinical trials. The following specific aims were originally proposed to achieve our long-term goal:

1. Design, fabricate and thoroughly test complete device systems (eg. catheter delivered stent and stent release mechanism with fluoroscopic positioning). The device designs include all facets of a clinical device including electronics, heating system (likely to be laser), microactuators and specialized delivery catheters. The device designs will be evaluated in functional, material property, thermomechanical properties, biocompatibility and thrombogenicity tests.

2. Functionally test the device systems through a series of experimental models. Three models are proposed that include a bench top tubing or excised vessel model, an anatomically correct femoral-to-cerebral vascular blown glass model, and an animal model.
3. Since SMP is a relatively new and undocumented material, we propose to optimize the material and mechanical properties of SMP for medical use. The clinically relevant thermomechanical properties of SMP are affected by the fabrication process, chemical and dye additives, water uptake, sterilization and device age.
4. Study and engineer the biocompatibility, biostability and thrombogenicity of the SMP devices.
5. Perform limited basic research for developing new SMP materials and/or biocompatible coatings.

STATUS OF RESEARCH AND PARTNERSHIP:
We are on track for all first year research goals set forth in the proposal. Development of both the clot extraction and polymer stent devices is progressing well. All of the project components including device engineering, materials development and characterization, biocompatibility studies and interventional studies are under way.

Some of the key studies have been building functional benchtop system, fabricating SMP microactuators, designing the optical fiber- SMP joint for both mechanical and optical criteria, building co-located thermal and optical microscope station.

A key clinical result of the device testing is that the coil must be able to be actuated to continually enlarge as the clot is extracted into larger and larger vessels. Otherwise, the clot can slip past the coil as the coil diameter becomes much smaller that the inner vessel diameter (0.75-0.5). The ability to continually actuate the coil to approximate the vessel ID has maintained the systems ability to withdraw the clot under flows and pressure much greater than physiological values.

As anticipated, a second key result was that at artery bifurcations, which are present in the silicon model of the neurovasular system that we use in the bench top design studies, the clot is flushed down the alternate arterial path as it is withdrawn past the Y-junction. Our solution is an expanding-tip micro catheter that captures the clot from the proximal side. The biocompatibility and materials studies are all underway and initial publications will be submitted in the second year of the project.

ISSUES:
There are no significant issues on the project. Initial delays in establishing regular communication among the participating scientists has been overcome by means of a twice-a-month, three-way televideo conferences between LLNL the UC Davis main campus and the UC Davis medical center in Sacramento. The televideo equipment and time has been kindly provided by the NSF Center for Biophotonics Science and Technology at UC Davis.

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GRANTING NIH INSTITUTE/CENTER: National Institute on Aging (NIA)

PROJECT TITLE: Morphological and Functional Musculo-skeletal Imaging

ABSTRACT:
Participants from the University of California San Francisco (UCSF), Lawrence Berkeley National Laboratories (LBNL) and Industry (Focus Imaging, Exponent Failure Analysis, General Electric) propose to form a Bioengineering Research Partnership (BRP) focussed on the systematic study of the morphology and function of the musculoskeletal system in disease and health. In addition, resources from existing research relationships with General Electric Medical Systems, SUN computers and IBM will be combined and utilized to rapidly evaulate and disseminate the developments of the BRP. The aim of this consortium is to improve medical care through bioengineering developments, and to facilitate close interactions between bioengineers, computer scientists, clinical investigators, basic scientists and corporate partners. This effort will expedite the development of clinically-relevant quantitative imaging tools and propel the technical advances from the laboratories into the operating rooms and clinics. We hypothesize that high resolution, fast magnetic resonance imaging techniques and positron emission tomography, combined with quantitative image analysis, processing and visualization, can provide new insights and clinically viable and relevant methods for objective evaluation of disorders of the musculo-skeletal system. The long-term objective of this partnership is to understand the link between morphology, function, biochemical changes and clinical symptoms in the musculo-skeletal system. An immediate objective is to develop, implement and optimize novel non-invasive imaging methods (magnetic resonance imaging: MRI and positron emission tomography: PET) that will allow us to depict the musculo-skeletal system, quantitate morphology, function, provide unique 3D visualization and graphical representations of function and morphology, as well as correlate these with biochemistry and c linical status. This research partnership is aimed at quantitating early degenerative changes in two clinical areas of emphasis: the knee and the spine. The first phase of the partnership will be technique development, followed by testing, and ultimately evaluation in case control studies in symptomatic patient populations. The specific goals are: (i) to develop quantitative morphological and functional markers for degenerative diseases of the spine, (ii) to develop quantitative morphological and functional markers for the degenerative changes in the knee and osteoarthritis.

STATUS OF RESEARCH AND PARTNERSHIP:
The immediate objective is to develop, implement and optimize novel non-invasive imaging methods (magnetic resonance imaging:MR and positron emission tomography:PET) to depict the musculo-skeletal system, quantitate morphology and function, and provide unique 3D visualization and graphical representation of function and morphology. The first phase of this

research partnership is aimed at quantitating early degenerative changes in two clinical areas of emphasis that have major societal impact: the knee and the spine. The BRP has expanded the imaging scope to include Fourier transform Infra-red Imaging, and High resolution Magic Angle Spinning (HRMAS). In addition the plans of extending combined Positron Emission Tomography, Magnetic Resonance, Computed Tomography to imaging the spine and knee have progressed considerably, and the proecess of execution has been fine tuned. The BRP has led to several accepted publications, abstracts and presentations at meeting. The goals of the BRP of integrating scientists from UCSF, clinicians and scientists at UC Berkeley and Lawrence Berkeley National Laboratories have proceeded as anticipated, leading to an integration of engineering and biomedical applications. In due course we have had to make changes to our operating procedures, and modified the methods of collaboration between sites at the administrative level. Confusion with different campuses have been mitigated through joint appointments. The significance of the partnership lies in the cohesive efforts of bioengineers, computer scientists, and clinicians in developing quantitative musculoskeletal imaging methodologies. The large number of publications and also the proposed RO1 applications in the next year make this is a fruitful and productive effort.

ISSUES:
The collaboration has been particularly fruitful with regards to graduate student involvement in multi-disciplinary research projects as well as clinician invo;vement. The interaction between the industrial componenet of the partnership have been deficient. There was no monetary contribution to the industria partners, perhaps that was the reason why with changing personnel, neither General Electric, Exponent have interacted at the minimum intellectual level. The third industrial partner based in France, with changing personnel, corporation names, was never inititiated into the partnership when it was started. However, Gregory Klein, who was involved at the LBNL componenet in the first and second year, now is in France, employed by a derivative of the corporation and initial enquiries about revitalizing the partnership has begun.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB)

PROJECT TITLE: Processing of Materials for Improved Biocompatibility

ABSTRACT:
The overall goal for this research partnership is to obtain fundamental understanding of a new process for sterilizing and cleaning biomaterials that results in clean, sterile, and functional biomedical devices. This project will provide the necessary science and engineering basis for evaluating cleaning and sterilization based on liquid or highly compressed carbon dioxide (CO2), and determining if the technology is more effective, less expensive, and more benign than technology based on steam, ethylene oxide, hydrogen peroxide, or radiation. The research is broadly applicable to the manufacture of biomaterials, implants, and prostheses. The research will support the development of the next generation of biomaterials (e.g. for tissue engineering) that are not compatible with current methods of sterilization and cleaning. This process has potential to eliminate material damage associated with irradiation. It will also eliminate the need for toxic sterilizing agents such as ethylene oxide, which tends to reside in polymeric materials.

Cleaning, particulate removal, and sterilization are currently separate steps that are crucial to the viability of medical devices. As medical implants grow more complex and as new biomaterials are developed for advanced applications, there is a crucial need to develop new techniques and processes that can clean and sterilize a wide variety of materials and devices at moderate to low temperatures, without introducing potential contamination, and without damaging the surfaces or otherwise compromising the biocompatibility or the functionality of the device. Surface damage can lead to premature mechanical failure and lack of biocompatibility. Failure to clean an implanted device thoroughly will lead to infection, inflammation, and reduced biocompatibility. Reduced biocompatibility in turn will encourage bacterial growth and infection. An improvement in biocompatibility could increase the life of a total hip replacement from the current 10 years (limited biocompatibility) to 20 years (ideal compatibility).

This Bioengineering Research Partnership project will address seven fundamental questions, namely: (1) What conditions produce complete sterilization? (2) At what conditions does CO2 remove soluble contaminants from biomaterials? (3) What are the mechanisms of CO2 sterilization? (4) At what conditions are particulates (i.e., metal or plastic residues of machining), microorganisms, endotoxins, and other cellular debris, removed by dense phase carbon dioxide-based fluids? (5) Can biomaterials be cleaned and sterilized effectively without damage to the

surface or material properties? (6)Are there limitations to the type of material or surface, or identity of the microorganism to be sterilized? How do specific material limitations affect the development of a CO2-based process? (7) What are the underlying physical, chemical, and biochemical mechanisms of cleaning and sterilization?

STATUS OF RESEARCH AND PARTNERSHIP:
Funding was initiated on July 15, 2003. Sterilization studies at South Carolina and at Clemson have focused on determining the process conditions where bacterial spores (B. subtilis, B. pumilis and B. stearothermophilus) are deactivated by supercritical CO2. Pure CO2 at temperatures up to 105C provides only modest (1 or 2 log) reduction in CFU for the bacterial spores investigated. Adding a small amount of water, however, greatly enhanced the effect of the supercritical CO2. With B. subtilis, the number of CFU could be decreased by up to 5 log at 80 C and at more extreme conditions (105C, 10,000 psia) all bacterial spore forms tested were completely deactivated in 25 minutes.

At South Carolina and the Medical University a protocol has been developed to determine the cleaning efficiency of supercritical CO2 for bacterial removal. S. aureus is cultured in vivo and individual bacteria are allowed to attach to polished coupons of titanium or stainless steel. These samples are then processed with CO2-based fluids and the degree of bacterial removal is quantified with fluorescent imaging, using propidium iodide stain.

We have also developed a protocol for removing endotoxins from metal surfaces. Films of commercially available endotoxin are deposited on coupon, processed with dense CO2 mixtures and the level of endotoxin remaining on the surface is quantified using the standard Limulus amebocyte lysate test. Preliminary experiments done with our collaborator, Thar Technologies, indicate that the use of additives may be necessary in order to remove the endotoxins from solid surfaces.

ISSUES:
The partnership comprises three universities, as well as one industrial partner and one external consultant. Communications, especially interaction between students and research scientists, were a concern initially but this has been resolved. The university researchers meet quarterly, rotating sites. PI Matthews has made one visit to Thar Technologies, and the consultant has visited two of the university sites, met with all investigators from the three sites, and provided a written review of the first eight months of the project. The project has generated several inquiries from industry, and the issue of FDA approval of this potential new sterilization process has been raised by industry. The BRP members will need assistance on this issue as the research progresses.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB)

PROJECT TITLE: Partnership for MR spectroscopic Imaging Data Processing

ABSTRACT:
Although MR Spectroscopic Imaging (MRSI) offers considerable potential as a diagnostic imaging technique, its use for clinical studies has been limited by complex requirements for data processing and analysis. Optimally, the data processing and analysis requires integration of a priori spectral and spatial information, including MRI-derived tissue segmentation, morphological analysis, metabolite MR parameters, and knowledge of normal tissue metabolite distributions. This Partnership will develop an integrated set of processing tools that satisfy these requirements, thereby simplifying implementation of MRSI for routine diagnostic imaging studies and increasing the potential information content.

This effort combines development of MRSI and MRI data processing under 5 projects located at 4 institutions, as well as data acquisition at multiple world-wide collaborative sites. Software will be developed for automated MRSI processing, tissue segmentation, brain region mapping, statistical analysis, and clinical presentation. Results from MRSI and MRI studies will be converted to standardized intensity units and transformed into normalized spatial coordinates, enabling the data to be pooled to form a database of MR-measured human metabolite values. This information will then be used to enhance statistical analysis of individual MRSI studies and map metabolite distributions in human brain. The resultant technical developments will be shared among several partners at collaborating medical research centers in the U.S.A., Europe, and Japan, where the package will be evaluated for diagnostic neuroimaging applications, with an emphasis on 1H MRSI of cancer, epilepsy, and neurodegenerative disease.

STATUS OF RESEARCH AND PARTNERSHIP:
In this first year, all partner sites required additional personnel recruitment, which is now complete. Software development has commenced and primary achievements include:
- A Web server has been established to provide: a) A central location for exchanging information, progress reports, software, sample data, and bug reporting; b) Software version control system (CVS) for all projects; c) Information for the general public describing this project and clinical applications of MR Spectroscopic Imaging.
- Definition of a hierarchical information management system to manage the multiple data sets obtained for each subject, which includes the acquired and processed MRI and MRSI data,

calibration data, and a record of all data processing. This system is based upon XML, an industry-standard protocol for which software tools are widely available.
- Definition of the data formats to be supported. To allow for support of MRS data (which is not adequately supported by any standard data format) from multiple commercial MR systems, all data will be "imported" into the processing environment using a common format for the parameter definitions, while still maintaining the data in the original data format.
- Each project has proceeded with definition of the functionality to be provided and with software development. This includes modifying existing modules to use the newly-defined XML study definition.

ISSUES:
The development of a central web site with message exchange facilities and software source code control is of considerable value in facilitating communication between researchers located across the country.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB)

PROJECT TITLE: Nano Arrays for Real Time Probing Within Living Cells

ABSTRACT:
The overall goal of this research is to exploit the recent development of rigid, vertically aligned, carbon nanofiber (VACNF) arrays to provide nanoscale electrochemical probes for mapping intra- and extracellular molecular events in and around living cells. VACNF are synthetic structures that self-assemble in a vertical orientation with respect to a planar substrate and that dimensionally span across multiple length scales, featuring nanoscale tip radii and lengths up to tens of microns. They may be deterministically synthesized on a variety of substrates (silicon, quartz, glass), with a high level of control over many parameters including length, tip diameter, aspect ratio, physical location on the substrate, and surface chemistry. In this effort, nanofiber probing arrays are being fabricated into devices that feature individually-addressable, nanofiber-based electrochemical electrodes where only the extreme nanoscale tip of the fiber is electrochemically active. The nanofiber serves both to elevate the electroanalytical measurement volume above the planar substrate (i.e. within and around cells as opposed to in-between the substrate and cellular matrix) and to electrically bridge between the nanoscale dimensions of the fiber tip and the microscale dimensions of the electrical interconnects of the substrate. Additionally, in this research effort, nanofiber-device fabrication approaches are structured around incorporation of microfluidic cell and analyte handling strategies, thereby providing architectures that will enable future high-throughput screening applications, such as clinical diagnostics of cell and tissue specimens and pharmaceutical exploration and discovery.

Research tasks are focused around several aims: fabrication of robust, nanofiber-based probing architectures; electroanalytical characterization of nanofiber-based electrodes against benchmark analytes as well as biologically-relevant species; investigation of cell/fiber interfacing schemes; and, ultimately, measurement of electrochemically-active species in and around cellular matrices. Reactive oxygen species (ROS; specifically hydrogen peroxide and superoxide anion) were selected as the target bioanalytes due to their critical role in virtually every aspect of cell function; (i.e. providing a source of metabolic energy from the oxidation of fatty acids, hydrolyzing complex organic substrates into reusable subunits, assisting in lipid biosynthesis, degrading harmful chemical byproducts, and acting as signaling and regulating molecules of cell growth and proliferation).

STATUS OF RESEARCH AND PARTNERSHIP:
Leveraging initial work conducted under the ORNL Seed Money program, a variety of studies were conducted to investigate nanofiber/cell interfacing. We have determined that successful fiber penetration into the intracellular domain requires nanofiber elements to be long enough to overcome cell membrane deformation (compliance) and to have conical aspect ratios that provided considerable mechanical strength at the fiber base. To provide such structures (30 nm tip diameters, 5-10 micron lengths, and base diameters of 500-1000 nm) our nanofiber growth process was modified such that individual fibers could be grown from photolithographically-defined, 500 nm diameter nickel catalyst dots [1].
Using nanofiber mediated reporter plasmid delivery and expression to indicate fiber/cell interfacing we have observed long term intracellular residence of nanofibers within cells (>22 days) via fiber-bound plasmid expression within those cells. Interfaced cells also grow and proliferate upon nanofiber substrates [2].
Based upon these cellular integration studies we have redesigned our probing device architecture to provide nanofibers of the appropriate size and mechanical strength to enable robust interaction with cell and tissue matrices. These modified structures, which feature 40 individually-addressable nanofiber elements within a 30 micron fluidic channel, have been fabricated using a process which depends only upon contact lithography for all lithographic processing steps. Additionaly, a similar architecture which features a more refined spacing of nanofiber elements (2 micron interfiber spacing which enables 4 probing elements within a single cell) is currently in fabrication. This new design exploits a new lithographic processing tool (400 nm resolution) that our laboratory has just brought online this month.
We have completed an analytical study of the electrochemistry of nanofiber devices to standard electrochemical analytes and determined the impact to this performance of microfabrication processing steps that we use during nanofiber-based device fabrication [3].

[1] AV Melechko, TE McKnight, DK Hensley, MA Guillorn, VI Merkulov, DH Lowndes, ML Simpson. Methods for Catalyst Particle Control for Large Scale Synthesis of Arrays of Vertically Aligned Carbon Nanofibers, submitted to Nanotechnology 14 Mar 2003
[2] McKnight TE, Melechko AV, Griffin GD, Guillorn MA, Merkulov VI, Serna F, Hensley DK, Doktycz MJ, Lowndes DH and Simpson ML. Intracellular integration of synthetic nanostructures with viable cells for controlled biochemical manipulation, Nanotechnology 14 (5), 551-556, 2003
[3] McKnight TE, Melechko AV, Guillorn MA, Merkulov VI, Doktycz MJ, Lowndes DH, Simpson ML. Effects of Microfabrication Processing on the Electrochemistry of Carbon Nanofiber Electrodes. Submitted to J Phys Chem B, 3 Mar 2003.

ISSUES:
It is with deep regret that we announce that one of our collaborators (Dr. Vladimir Merkulov) suffered severe brain trauma during this FY. Dr. Merkulov's contributions are significant in the field of nanofiber synthesis, and his absence from this project and from our daily lives is deeply felt. We wish Dr. Merkulov the best with respect to continuing recovery from this condition. In his absence, his colleague, Dr. Anatoli Melechko has provided us wonderful support with nanofiber synthesis and device integration.

We are pleased to announce that our partnership is expanding to two new academic institutions, as Dr. Stephen Jacobson and Dr. Christopher Culbertson become faculty members at Indiana University and Kansas State University, respectively. We are excited about the microfluidic aspects of our research expanding to these two fine institutions.

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GRANTING NIH INSTITUTE/CENTER: National Insitute of Child Health and Human Development (NICHD)

PROJECT TITLE: Molecular transmission of force in the CNS

ABSTRACT:
The partnership is designed around three central areas: cellular mechanics, molecular measurement technologies, and cellular informatics. These areas are integrated and applied to the study of traumatic injury to the CNS. Our main objectives are to develop the appropriate technological infrastructure to study, at the single cell level, the heterogeneity of events that occur within a traumatically injured neuron. We propose that a unique set of molecular events at the transcriptional/translational level, mediated through immediate biochemical changes in the cell, will direct the fate of individual neurons in the traumatically injured brain. The vast extent of information available for identifying these unique characteristics is significant, and will require an informatics based approach to decipher the important events from the less significant changes in the cell. In addition, we need to expand and develop moderate throughout technologies to accommodate more ready screening of compounds to test for therapies in the injured brain. We focus on the changes that occur with apoptotic and necrotic cell death in the cortex, using a combination of in vivo and in vitro models in identifying the appropriate targets for intervening to repair neurons in the brain after injury.

STATUS OF RESEARCH AND PARTNERSHIP:
In the area of cellular mechanics, in vivo models of traumatic brain injury have been adapted for in vitro study, and efforts in the past year have developed computational estimates of how cellular and subcellular structures in CNS tissues deform under traumatic loads. Models have been developed for white matter tissue, and data is being generated for similar structurally based representations of gray matter tissue. From these models, we have defined the mechanical environment experienced by cells in CNS tissue during traumatic loading, and have completed in vitro studies that identify the primary mechanosensitive pathways in cultured hippocampal and cortical neurons. These provide important upstream signaling information for developing a perspective on specific gene expression profiles generated within traumatically neurons, as well as developing potential therapeutics.

For molecular measurement technologies, work in the past year has focused in two areas. First, we have applied previously developed technologies to isolated mRNA from individual neurons within the traumatically injured brain and developed a specific gene expression profile for neurons isolated from the injured brain. Results show there is a unique subset of genes that are differentially expressed in cells undergoing apoptosis relative to healthy neurons, and these

changes persist over the first 48 hours following trauma. In the second area, investigators have developed a new approach, termed antibody positioned RNA amplification (APRA), which allows one to further discriminate and target a subpopulation of genes within the neuron. With this technique, it is now possible to selectively separate and amplify a subpopulation of mRNA that is located within close proximity to a protein identified by a specific antibody. Work is now progressing to use this technology to identify the subpopulation of mRNA attached to motor proteins (e.g. kinesin, MLKP1) in the neuron after injury. Our aim is to seek out which mRNA species are differentially associated with motor proteins after injury, with the goal of using this information to better target possible therapeutics.

In the area of cellular informatics, we have two specific focus areas. First, we are using both empirical and computational representations to scan neuronal populations and detect if there are specific subpopulations of neurons that are specifically vulnerable to either mechanical or chemical insults. For example, we have discovered that neurons responsive to GABA stimulation in culture are relatively insensitive to a mechanical stimulus; the intrinsic electrical activity that is operative at the moment of mechanical injury also has a significant impact on the neuronal response after injury. For the second foci area, we are using the bioinformatics investigators to look at the temporal changes in gene expression following chemical or mechanical stimulation. We are generating material for analysis over a cluster of timepoints to generate models of related gene families that are differentially changing over time following this stimulation.

ISSUES:
Within the three major foci areas defined above, there are significant issues that appear for the upcoming year. We need to scale-up some existing technology for potential medium throughput analyses, and to scale down some current molecular technologies to probe the mRNA localization and transport at the single cell level. First, we are now piloting a system to provide a moderate throughout tool to screen compounds for in vitro traumatic injury. The system will be adaptable for evaluating fluorescence based measures of cell population changes, and will considerably increase the capacity for testing efficacy in vitro. Second, we will continue to work on the APRA technique and focus on improving the ability of the technique to isolate subpopulations of mRNA within clusters of individual cells. Third, we are developing molecular beacons to target specific mRNA species within the neuron and track the changes in these subspecies after chemical stimulation. This will develop a new level of information that will be incorporated into the cellular informatics group activities.

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung and Blood Institute (NHLBI)

PROJECT TITLE: New Approach for the Treatment of Asthma

ABSTRACT:
The goal of this proposal is to further develop and evaluate an innovative and potential clinical treatment for asthma. Although there are a multitude of different possible triggers, an acute asthmatic attack is always characterized by contraction of the smooth muscle in the airway wall. Despite this common end point, most of the clinical asthma research and therapies in recent years have focused on understanding the immunologic factors that often lead to asthmatic attacks. In contrast, the present proposal describes research and development focusing on a treatment that will chronically impair the ability of smooth muscle to contract, and will thus be effective in treating asthma regardless of the initial trigger or intermediate pathways. The work involves a close working partnership between the physiologic laboratories and expertise at the Johns Hopkins University and a biomedical engineering company, Broncus Technologies, that is providing the mechanical and bioengineering skills needed for product development. As a means of treating asthma, Broncus Technologies, in collaboration with leading clinicians in the field of asthma, is developing a biomedical device system known as the AlairTM System (Pat. Pend.). The idea currently under development involves delivering heat to the airway wall in a precise and controlled manner, such that the contractility of a significant fraction of the airway smooth muscle, in airways as small as 3 mm, is eliminated. The process for this treatment has been termed bronchial thermoplasty. The partnership established between Broncus Technologies and Johns Hopkins will facilitate the functional testing needed for this device development. In the long term, this partnership will help ensure the smooth translation of this new concept and device to the clinical arena.

STATUS OF RESEARCH AND PARTNERSHIP:
Results in the second year of this partnership have focused on experimental work using high resolution CT imaging to examine the responsiveness of airways treated with the current prototype device that delivers energy to the airway wall through a bronchoscope. To this end, Broncus has developed a custom R&D software platform for the RF Generator, and a device with more user control for animal experimentation. Work at Broncus has also been initiated on theoretical modeling of the heat transfer in the airway wall. One of the most puzzling early findings is the fact that the histologic observations of smooth muscle damage don't seem well correlated with the location of the source of the thermal contact points. The predictions of this model will be correlated with existing and new histologic findings.

Experimental work at Johns Hopkins in the past year was concerned with two ongoing experiments. In results presented at the 2003 ATS meeting, we have now confirmed that airways

treated with the device do not contract to as small an area either at baseline or with any dose of inhaled agonist. We also have preliminary data showing that the distensibility of the treated airways may be increased over untreated airways. This effect may correct the abnormal contractile response of asthmatic subjects to deep inspiration into the normal dilation.

ISSUES:
None.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Neurological Disorders and Stroke (NINDS)

PROJECT TITLE: MR Image Analysis: Identification of a Surrogate

ABSTRACT:
Multiple sclerosis (MS) is the most common demyelinating disease in humans and has a complex clinical course that includes unpredictable relapses and variable remissions. This makes clinical evaluation of MS difficult. Therefore, current clinical trial designs must incorporate large numbers of patients followed over long periods. These designs are expensive and may deprive patients timely access to effective treatment. The use of robust surrogate marker(s) that have predictive value could reduce problems in evaluating new drugs and improve the management of individual patients. MRI-based measures such as volumes of lesions, black holes, contrast enhancements, atrophy, and magnetization transfer ratios, are expected to serve as robust surrogates. However, a number of studies have shown that the correlation between these MR measures and clinical score is weak. We hypothesize that this weak correlation is in part due to the use of improper image analysis tools necessary for robust image quantitation and in part due to a failure to define the correct MRI surrogate. In these studies we propose to develop an integrated image analysis package that is robust and automatic for accurate quantitation of tissue volumes. An important feature of this analysis package is its ability to analyze images acquired on a wide range of MR scanners using a plethora of MR sequences, greatly extending its utility. This package allows us to follow temporal changes in individual lesions, as well currently used global changes. This analysis package will be rigorously evaluated using an extensive database that contains images on more than 1,500 MS patients, followed over several years. Using this database, we propose to identify surrogate(s) based on individual or some combination of MRI-measures. Finally, this software will be distributed to a few select centers for multicenter evaluation. While the main emphasis is on MS, this system should be readily adaptable to investigate and manage various neurological disorders that require accurate determination of tissue volumes and their temporal change.

STATUS OF RESEARCH AND PARTNERSHIP:
We have initiated work on 1) retrospective image registration and 2) image segmentation. With our industry partner, we have also started porting the code from UNIX to PC platform.
a)Retrospective Three-Dimensional Image Registration: We have combined differential evolution (DE) with DIRECT (dividing rectangle) for global optimization of mutual information (MI) for retrospective 3D image registration. Our simulations clearly demonstrate that combining DE and DIRECT yields a lower cost function, and hence better registration than using DE alone. Application of this technique to the multi echo MR images indicates that the calculated rotations

and translations were within 0.2% and 0.1%, respectively. This is a completely automatic technique.
b) Segmentation: We developed a novel 4D feature map based multispectral methods for segmenting MR images. This is based on “divide and conquer” method that allows the generation of feature maps in higher dimensions on regular PC’s. In addition, a pre-classification procedure is adopted for reducing the number of points in the feature space that need to be classified with the KNN method. This considerably reduces the computational complexity. c)Partnership with Biotechnology Consulting & Research : In partnership with Biotechnology Consulting and Research, Irvine, we have initiated porting the code from UNIX to PC-based Window platform. The Windows version is being written in Microsoft Visual C++ 6 using Microsoft Foundation Classes (MFC). The multiple document interface (MDI) of MFC provides a framework of application, document, and view objects for the management of multiple images and files.

ISSUES:
The work is progressing as planned. Currently, there are few issues with the partnership or research that deserve special attention.

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute (NHLBI)

PROJECT TITLE: MAGNETICALLY SUSPENDED ROTOR BLOOD PUMP 1 R01 HL 643-04

ABSTRACT:
At the onset of this grant, the objective was to develop a magnetically suspended centrifugal flow pump to support adult cardiac failure patients with an average flow of about 6 liters/min and a developed pressure of 100 mm-Hg. The impeller of this pump has five blades, is 47 mm diameter and is magnetically levitated. The fourth and final prototype, called CF4, has been successfully tested in mock circulatory loop tests and in a number of animal tests to date. The pump has been successfully implanted in a number of calves with the longest duration being 112 days. No hemolysis was observed during any of the tests. The design has been frozen by one of the partners, MedQuest Products, Inc., and funds are being sought for commercialization of the LVAD.

For a long time, Dr. Don Olsen has been very interested in developing a more compact magnetically suspended axial flow pump design. The centrifugal pump design presents a geometry that is not easily implantable in humans because of the inflow and outflow cannulas. Also, there is a secondary flow behind the centrifugal impeller that may promote thrombosis. In contrast, because of the small size and tubular construction, the axial pumps have a better anatomical fit. Thus they require less time to implant, thereby decreasing the cost and invasiveness of the procedure plus allowing for better long term biocompatibility and reliability. A new axial flow pump design of this type was initiated by the BRP team about one year ago. The engineering development program provided new insights into magnetically levitated pump design and computational fluid dynamics (CFD) analysis for blood flow path design. With this basis of expertise, a new, smaller axial flow pump has been designed. The new pump has an unobstructed blood flow path that is superior to that of other pumps being developed, and the axial flow design allows for superior anatomical fit. This data permitted the design and miniaturization of the magnetic bearings. The experimental results are in excellent agreement with the predictions based on computational fluid dynamics modeling.
The magnetic levitation uses permanent magnets for axial thrust and a combination of permanent and actively controlled electromagnets for radial centering. This arrangement allows a blood flow path with no obstacles. In addition to allowing for the design of a superior blood flow path, the magnetic levitation will provide pump lifetime on the order of 15 years, and, hence, the Olsen VAD is targeted as destination therapy as well as bridge applications..

The new pump has been named the Olsen VAD and the first prototype has been built of plastic to allow flow visualization, pump performance measurements and measurement of fluid forces on the impeller. The first implantable versions of the pump will be manufactured by August, 2003 and animal testing will begin shortly after characterization on a mock loop. The new Olsen VAD will be the object of a new BRP grant proposal.

STATUS OF RESEARCH AND PARTNERSHIP:

There are several major objectives that must be met to make the new and innovative axial flow VAD design acceptable: there must be a very clean and straight through blood path, the pump impeller and stationary vanes must be designed for high efficiency and manufacturability, the magnetic suspension must be very compact and require only very low power, and the pump must be biocompatible. The new Olsen VAD prototype pump with these characteristics has been completely designed and 10 prototype units are currently under construction.

One of the best features of the Olsen VAD is the straigth through blood path which does not require any secondary blood flow paths. The flow enters the inlet region axially and passes through the axial flow impeller passage and then out through the diffuser. The direct flow path is possible because of the innovative magnetic suspension system described below.

Computational fluid dynamics modeling was used to develop the blood flow path and predict the pump performance and fluid forces on the impeller. The fluid shear forces were also calculated to provide guidance for avoiding hemolysis and thrombosis. A manufacturable design has been completed.

The magnetic suspension system consist of an axial permanent magnet to withstand the axial impeller forces, one radial permanent magnet radial bearing and one active magnetic bearing. Special new permanent magnet designs, using no power, are configured to allow for a perfectly straight through axial flow path. All blood contact surfaces are made of pure titanium for blood compatibility.

ISSUES:

The partnership between The PI and the UTAHI, and the team at the UVA continued strong and very productive throughout the funded period and sufficient advancement towards a superior designed VAD has been made that a new BRP proposal is to be submitted. The initial VAD proposed and funded was for a centrifugal flow pump with the impeller suspended in magnetic bearings. This design was developed and the bench testing was excellent and several calves were implanted with outstanding results with particularly the later prototypes, CF4VAD. These calves experienced no hemolysis for as long as 112 days and at device retrieval analysis, demonstrated no impeller touchdown thus proving very robust magnetic bearings. The success was so outstanding that MQP elected to freeze the design and seek money for commercialization of the centrifugal flow VAD. They have elected to not be involved in the newly designed axial flow pump with the markedly improved blood flow path and external geometry for improved anatomical fit at implantation.

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Pending: Wilson-Greatbatch, Inc. (Clarence, N.Y.)

GRANTING NIH INSTITUTE/CENTER: National Institute of Neurological Disorders and Stroke (NINDS)

PROJECT TITLE: Development of Networked Implantable Neuroprostheses.

ABSTRACT:
Neuroprosthetic devices that electrically stimulate paralyzed muscles provide functional enhancements for individuals with spinal cord injury and stroke such as standing and stepping, reaching and grasping, and bladder and bowel function. Current implanted neuroprosthetic systems utilize considerable external powering and signal processing, and each system is tailored to the specific application for which it was intended. The need to design a customized implant system for each application severely limits progress in the field and delays introduction of new technology to the end user. Therefore, we are developing an implanted neuroprosthesis having an open architecture that is easily configured for current and anticipated neuroprosthetic applications, allows new innovations by various participants in the field, minimizes external components, is implanted using minimally invasive techniques, and can be clinically implemented.

To satisfy these requirements, our Biomedical Research Partnership (BRP) project is developing a networked neuroprosthetic system (NNPS). The NNPS is based on a network of small implanted modules, distributed throughout the body, and linked to a centralized power source. A variety of modules will be developed, each with a specific function including: muscle-based stimulation, nerve cuff stimulation, biopotential (electromyogram, electro-oculogram, electro-encephalogram, electroneurogram) signal recording, body segment orientation measurement and acceleration measurement. Other potential modules that could be incorporated into this system include mechanical actuators, joint angle transduction, and strain gage based sensors.

STATUS OF RESEARCH AND PARTNERSHIP:
Our partnership was established in October 2001 and we have made significant progress in the first 20 months of the currently funded BRP project. Our progress includes establishing the overall network topology, identifying the network communication protocols, identifying all system components and their operating environments, and starting bench-top proof-of-concept testing.
We have evaluated various configurations of networked structures, and have selected our primary network topology. The selected network topology consists of an internal network based on a central power module with four power-transfer/data-communication multi-drop backbone leads, thus providing a scaleable network infrastructure for connecting multiple actuator-type and sensor-type modules. The infrastructure will support totally implanted closed-loop systems, an important goal of the distributed system concept.
We have evaluated various network communication protocols, and have selected the Controller Area Network (CAN) protocol as the internal network communication protocol. This approach leverages a robust, reliable and proven real-time protocol immediately. This approach also leverages sophisticated and

powerful integrated circuit hardware immediately and positions ourselves to benefit from advances in technology that will transpire over the lifetime of our technology. The CAN implementation provides an open interface standard for others to utilize, an important goal of the distributed system concept.
Based on the network topology and communication protocol, we have defined all system components and their operating environments. Key system components include the Access Port, Network Segment Cables, and distributed Actuator/Sensor Modules. The Access Port provides power from a rechargeable lithium-ion battery and connects up to four Network Segment Cables, it can forward messages or parts of messages between segments. The Network Segment Cables form a multiple backbone network for distributed module inter-connectivity. Each Actuator/Sensor Module contains an microcontroller providing a network interface controller and local processing capability. These features implement the communication protocol and minimize the communication rate between modules. The microcontroller is in-system-programmable, an important goal of the distributed system concept.
We have identified the following four FES operating environments that must be addressed and accommodated by the design implementation. They include the surgical, clinical, user and research environments. The surgical installation environment limits access and communication methods. The clinical programming environment must provide transparent real-time access to all user implanted components. The user environment must have robust non-tethered operation. The research environment requires high-bandwidth communication and processing. The challenges of each environment are providing opportunities for defining and optimizing their interaction.
Important proof-of-concept testing has started to demonstrate technical feasibility and realization. A single segment bus implementation has been implemented to develop and test communication messaging and hardware designs. Optimized network interfaces have been simulated and will be validated with surface mount implementations in the coming weeks. The simulations to date verify a two-wire interface design that will significantly lessen the burden on other system issues, an important goal of the distributed system concept.

Our current grant period will end in August 2003. A renewal proposal was submitted in January 2003 which is currently pending review. In the renewal proposal, we intent to fabricate various NNPS components and perform complete in-vitro and in-vivo device qualification testing. We will assemble components to produce a complete system, including a stimulator module to produce the necessary output for muscle-based electrical stimulation, and a sensor module that records and processes myoelectric signals. In the later years of the proposal, we intend to realize a first configuration of the NNPS in individuals with spinal cord injury to provide enhanced grasp/release. This human feasibility study will provide the foundation for broader clinical application of the NNPS.

ISSUES:
Our primary issue remains managerial rather than technical. We feel that we have made substantial progress in the research. However, we have encountered delays and problems when attempting to exercise flexibility in changing existing and establishing new contractual relationships with outside industry. An improved ability to quickly and freely team with industrial partners and form more dynamic working partnerships would greatly enable us to evaluate existing technologies and investigate new ones.
Clearly communicating the intended flexibility of the NIH-BRP program to University administrative officials – when dealing with subcontractors – would streamline our ability to make changes in consortiums and their related budgets, and thus improve our efficiency and speed progress.

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GRANTING NIH INSTITUTE/CENTER: National Eye Institute (NEI)

PROJECT TITLE: Engineering Approaches to Low Vision Rehabilitation

ABSTRACT:
This project applies novel engineering approaches to the problems of low vision rehabilitation. We are building prototype devices based on solid theoretical foundations that, eventually, will become marketable rehabilitation products. The devices, designed and built with the help of our engineering partners, will be tested critically using diverse patient populations, with the help of the clinical partners, to determine the effects on function and on the quality of life.
We are developing and testing both optical and electronic devices that implement three specific engineering approaches aimed at restoring (at least in part) the important interplay of central (high-resolution) and peripheral (wide-field) vision: multiplexing: dynamic control of display: and image enhancement. Also, we will show that various combinations of these approaches are possible and likely to be beneficial. In our assessment and testing we emphasize two approaches: a virtual environment for controlled and quantitative testing in the laboratory, and on-the-street evaluation for real-life determination of the effect and usefulness of the devices and techniques.

STATUS OF RESEARCH AND PARTNERSHIP:
The project has two major components: device development and device evaluation. Both components have been progressing well. A paper describing implementation of multiple prototypes of the electronic device, augmented-vision for monocular restricted peripheral visual field, have been published. The second generation of the HMD was delivered and underwent testing. Design of the third generation device was specified and system is under development; the portable edge detection (monopolar) devices have been delivered. A bipolar version of the device needed for the dynamic control of display is in final stages. Another novel optical device implementing multiplexing was invented and a patent application submitted. Studies of eye movements while watching TV, needed for the dynamic control of displays, are underway. The virtual mall is in place and working. A paper on the first study is in final preparation, a second study was completed, and a paper on the calibration system is almost ready. Real walking studies with two devices are underway and have completed testing half the subjects. On-the-road driving studies are under way in Holland and AL and in design in RI. The first driving simulator study is under development in the Boston VA. Two papers on the enhancement projects were published in IEEE journals.

In the last year we have published 8 journal papers, 2 conference proceedings, and have been awarded a patent. We have presented numerous conference papers including invited and award presentations. An active web site serves both for internal project communications and dissemination of information.

ISSUES:

To solve the problem we had with the National Advanced Driving Simulator (NADS) in Iowa, we formed a new partnership with the Center for Innovative Visual Rehabilitation at the VA Med. Ctr. in Boston. For the project they have purchased a driving simulator and our first study is being developed on that simulator.

The MA Department of Motor Vehicles did not approve the on-the-road driving studies. So, we formed a new partnership with the group from Groningen, Holland and the hemianopia driving study with our prism treatment is currently underway there. Another partnership forged with the Dept of Ophthalmology at the Univ. of Alabama at Birmingham has resulted in a driving with restricted peripheral field study currently running there. A driving with bioptic telescope study was approved in the state of RI and the design of driving courses there is underway.

We have a formed a new partnership with Chadwick Optical from Vermont (in place of the Swedish partner, Multilens). This partnership led this year to a recently awarded SBIR grant on a novel design of the hemianopic prisms. A study is now underway.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Deafness and Other Communication Disorders (NIDCD)

PROJECT TITLE: Micro-electric impedance spectroscopy (μEIS) of hair cells

ABSTRACT:
This project is aimed at the development and testing of micro-electric impedance spectroscopy (microEIS) and tomography (microEIT) hardware and reconstruction software to record and image the spatio-temporal distribution of electrical properties within the cytoplasm, organelles and membranes of vestibular and auditory sensory hair cells. A combination of flex-circuit technology and standard lithographic microfabrication techniques are used to construct micro-recording chambers instrumented with arrays of metal electrodes at subcellular dimensions. Isolated cells are positioned within the instrumented recording zone under mircroscopic observation and interrogated using radio frequency electrical signals. Voltage and current are measured around the outside surface of the cell and used to reconstruct three-dimensional maps or images of the conductivity and permittivity throughout the cell. micorEIT systems are being used to interrogate electrical properties of cochlear outer hair cells and type II vestibular hair cells in response to micromechancical stereocilia displacements, electrical stimuli, and chemical/neurotransmitter stimulation. Results are contributing to our fundamental understanding of the spatial distribution and temporal response of electrical properties in these important sensory neurons. Perhaps more importantly, microEIT devices developed as part of the research, are providing an entirely new window through which to view the living machinery of a wide variety of normal and pathological cells. The project integrates bioelectricity, imaging, bioinstrumentation, micro/nano-bioesensors, physiological modeling/computation, biomechanics and microfluidics. Devices involve on-chip transport of solutions/pharmaceutics and living cells.

STATUS OF RESEARCH AND PARTNERSHIP:
The project is currently in the 20th month of funding (1 RO1 DC04928-01, start date: August 2001). All subcontracts were established within the first month of the grant. With minor exceptions, the project is proceeding as outlined in the proposal. Experiments using simple two-electrode μEI devices with saline solutions and cells have provided additional data and have contributed to incorporation of an on-board, RF, computer-controlled bridge circuit in the 2nd generation devices. We have completed several types of 2nd generation μEI platforms and have had success in recording spatio-temporal electrical properties of isolated hair cells. Due to the small scale of the devices and high interrogation frequencies employed, considerable attention has been devoted to the development of reliable, user friendly, microfluidic and electrical interconnects. The μEI devices are interfaced to a bank of computer controlled arbitrary

waveform generators, digital scopes, and lock-in amplifiers. Measurements are routinely made over a range from 100Hz -30MHz. Experiments with cochlear outer hair cells have revealed a very unusual piezoelectric behavior of the membrane. This includes a resonance in the ultrasonic range. We have also been successful in measuring charge movement in the lateral wall of the cochlear outer hair cells at temporal resolution far exceeding that possible with pipette-based recording technologies. These data are contributing to our understanding of the role of piezoelectricity, the protein prestin, and nonlinear capacitance modulation in outer hair cells ? biophysical events fundamental to outer hair cell somatic electromotility and the exquisite selectivity and sensitivity of the mammalian cochlea. We have also used the technology to measure changes in cardiac myocyte membrane conductance during contraction ? changes assocated with opening and closing of ion channels. These measurements clearly demonstrate the utility of the method to noninvasively interrogate excitable membranes of isolated cells. The technology has also enabled experiments in directions completely unexpected at the time of grant submission. For example, we have been able to record time-resolved changes in membrane RF impedance during the process of electroporation. These data represent are the first with sufficient time resolution to resolve both opening and closing of individual pores and has led to a new working model of the process. We have also been successful in delivering macromolecules to isolated cells using microchamber devices.

ISSUES:

We have not experienced any serious administrative or technical issues. I do have several concerns regarding IP, technology transfer, and contractual agreements between institutions and industry. Our research is primarily driven by specific scientific questions ? questions which require the development of new technology to answer. We are very excited about the technology and have been making very rapid progress on the engineering side. Applications of our technology are much broader than we initially envisioned and we find interesting questions pulling from all directions. It is indeed exciting to work in an area with such a broad potential for impact on health and the human condition. The PI is concerned, however, that efforts by the team members in the area of technology development may dilute the time available to devote to the key scientific questions at hand. This is a particular concern since peer review is often dominated by the science and not the engineering.

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute (NHLBI)

PROJECT TITLE: Engineered Cardiac Morphogenesis: Stem Cells and Scaffolds

ABSTRACT:
The long term aims of this project are to produce tissue engineered ventricular wall patches for myocardial repair, ventricular assist devices, and eventually replacement ventricles. Our team has expertise in biomaterials, bioreactors, tissue biomechanics, embryonic and somatic stem cells, muscle development, vasculogenesis, extracellular matrix, cardiac injury and regeneration, animal and human heart transplantation. This team will collaborate across three research foci: 1) "Instructive" tissue scaffolds. Advanced biomaterial fabrication will be used to engineer biodegradable matrices and meshes with controlled pore dimensions, modified with receptor specific molecules. Matrices will be optimized to instruct cell attachment, orientation, migration, proliferation, differentiation, and overall tissue organization. 2) Cell and developmental biology. Primary and stem cell-derived muscle and vascular cells will be studied on modified scaffolds to determine the optimal conditions for producing functional muscle tissue and vascular networks. Engineered tissues will be subjected to mechanical stresses to direct maturation toward in vivo phenotypes. Bioreactors will be developed to implement these requirements on a useful scale. 3) Clinical science and animal models. Contractile ventricular patches will be tested in an injured heart model. Integration with host tissue and restoration of contractile function will be evaluated. A tubular cardiac assist organ comprised of vascularized myocardium and endocardium will also be developed. The "tube hearts" will be conditioned in pulsatile flow circuits, assessed for mechanical performance in vitro, and eventually grafted into aortas of syngeneic rats for in vivo evaluation. Progress toward these goals should establish design principles necessary for constructing more complex ventricular devices

STATUS OF RESEARCH AND PARTNERSHIP:
This BRP comprises seven major laboratories at the University of Washington, two at the Hope Heart Institute, one at the Univ of Toronto. The program is just now entering its fourth year of funding.
Status of the Partnership: The partnership remains active and robust. The UW laboratories are highly interdisciplinary and include units from both the College of Engineering and the School of Medicine. The participation of the Hope Heart Institute continues to be strong. Extramural partners continue to willingly expend the time, effort and money to travel to campus for strategic planning meetings and research activities. Changes in the partnership have arisen as a result of the dissolution of Advanced Tissue Sciences, Inc. previously a corporate partner. Work has been concentrated in the UW laboratories and one new investigator will be added to focus on chitosan-alginate composite materials.

Status of the Research: We have continued comprehensive studies of mouse ES cells seeded and cultured on a variety of scaffold materials. The lab has acquired training and experience with human ES cells and is preparing to extend the scaffold studies to include hES cells pending the outcome of a recently submitted supplement application. In recently published work we have reported on the engineering of arrays of microvessels that may be used to provide perfusion of nutrients in larger heterogeneous constructs. We have previously demonstrated control of cell proliferation in skeletal muscle cells using an engineered FGF receptor chimera. This system is now being applied to endothelial cells.

ISSUES:
The unexpected departure of our industry participant presented fiscal and some research challenges. Synthesis of individual projects remains challenging.

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GRANTING NIH INSTITUTE/CENTER: National Institute on Drug Abuse (NIDA)

PROJECT TITLE: High Field MR Research in Drug Abuse: A Bioengineering Research Partnership

ABSTRACT:
Magnetic resonance spectroscopy (MRS) and functional magnetic resonance imaging (fMRI) are extraordinarily promising new imaging modalities that are increasing our understanding of the nature of drug abuse and addiction. In March, 1999, the Office of National Drug Control Policy (ONDCP) and McLean Hospital agreed to jointly fund a Varian NMR Systems 4.0 T MR scanner which will be dedicated to substance abuse research at the McLean Brain Imaging Center.

The present BRP application describes a series of ten engineering projects which will enhance the capabilities of this unique magnetic resonance research center to conduct studies of individuals with substance abuse disorders. This research program will involve bioengineering and clinical investigators at McLean Hospital, the Beth Israel Hospital, Tufts University, Boston University, the University of Washington, the University of Oxford, the University of California, San Francisco, and Wayne State University.

Specific projects are summarized below:

1. Objective motion detection and correction in time series fMRI experiments.
2. Optimized phased array coil design.
3. FMRI image registration and signal dropout reduction in brain regions with high susceptibility effects.
4. Functional T2 relaxometry of brainstem and midbrain monoaminergic nuclei.
5. Estimation of cerebral blood flow and volume using dynamic susceptibility contrast MRI.
6. Proton echo-planar spectroscopic imaging at 4 T.
7. Two-dimensional, proton magnetic resonance spectroscopy of amino acid neurotransmitters.
8. Statistical methods for assessing drug effects and confounds in MRS and fMRI studies.
9. Concurrent, high resolution optical imaging and fMRI.
10. Concurrent EEG and fMRI assessment of drug-induced alpha wave activity.

All of the projects listed above have been have been designed to address technical limitations encountered in the course of conducting NIDA-funded clinical imaging studies at 1.5 T field strength. Importantly, funds requested for this BRP will be used exclusively to support the engineering aspects of the research projects.

STATUS OF RESEARCH AND PARTNERSHIP:

1.Our BRP grant was funded by NIDA, with a substantial budget cut, effective 1 September 2001. Of the ten projects listed above, one (#8) was eliminated and two (#2 and #3) were combined. The remaining 8 projects all had significant budget cuts, based primarily on the review of the grant proposal.

2.The Varian Unity/Inova 4 T Scanner was installed at McLean Hospital in May, 2001. Many unresolved issues reported last year (scanner quenching, patient table, sound dampening) have been resolved. Remaining issues include: software and filters for decoupling, EPI stability and ghosting, and user interface.

3.Additional funding to support hardware purchases was obtained from the Counterdrug Technology Assessment Center (CTAC) of the Office of National Drug Control Policy (ONDCP) to expand the scope of the work that we could do within the BRP. Equipment has been ordered and some has been delivered. Work on projects #9 and #10 has been delayed due to long lead times for equipment delivery.

4.We have performed several upgrades to the 4 T scanner, including installing a higher power RF amplifier to improve spectroscopy performance, obtaining a new shim amplifier to enable dynamic shimming, and developing improved reconstruction software in-house.

5.A research agreement was established with Qualisys Medical to supply two near infrared, high resolution cameras for project (#1), improving resolution tenfold over our previous cameras.

6.Methods for fully implementing 2D MRS on the 4 T scanner have been reduced to practice (project 7).

7.Three new areas of research have been identified: carbon-13 MRS studies of cerebral metabolism (in collaboration with investigators at Yale University), biological effects of magnetic stimulation, and sodium-23 MRS studies of cerebral membrane properties.

ISSUES:

None.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB)

PROJECT TITLE: Polarization Sensitive Optical Coherence Tomography (PSOCT) for Glaucoma Diagnosis

ABSTRACT:
The goal of our project is to measure depth resolved birefringence of the retinal nerve fiber layer (RNFL) and document birefringence changes that occur with glaucoma. We have constructed two PSOCT systems to measure depth resolved birefringence of the RNFL: an open-air PSOCT system at UT Austin and a fiber-based system at Massachusetts General Hospital. The fiber based PSOCT system is coupled to a slit lamp for clinical measurements. A typical measurement of phase retardation/unit depth (PR/UD) in the retina between the fovea and optic nervehead of a human volunteer at Massachusetts General Hospital is 0.3 degrees/micron.

The primary focus of our research effort over the past year has been the creation of phase retardation maps of the primate retina and measurement of depth-resolved phase retardation in human glaucoma patients. At the beginning of the project we expected to use RNFL birefringence as an inherent tissue property to enhance measurement of retinal nerve fiber layer thickness (RNFLT). We found that PSOCT intensity images give accurate RNFLT directly without using any phase information. Phase retardation maps provide a second and independent measurement of the RNFL health. Moreover, the phase retardation/unit depth (PR/UD) of the RNFL provides a clinically important measure of the nerve density. Thickness and PR/UD provide two independent measures of the RNFL and are being recorded and analyzed in human and primate studies.

Both PSOCT instruments were calibrated using mica plates and tissue phantoms. An algorithm was developed to reduce speckle noise and calculate phase retardation and optical fast axis orientation from computed Stokes vectors using two incident polarization states. A collection of registered B-scans of the peri-papillary retina produces RNFLT and PR/UD maps.

Analysis of PSOCT images of the cynomologous primate retina indicate PR/UD varies with lateral position on the RNFL. This important observation implies that RNFL thickness and phase retardation are independent measures and RNFLT cannot be determined from phase retardation without knowing the PR/UD at a specific retinal position.

A differential phase optical low coherence reflectometer has been built that can resolve one milliradian or an optical pathlength difference of one angstrom. This resolution is theoretically

sufficient to image the birefringence change produced by an action potential on a single neuron with signal averaging. Experiments using the giant squid axon are being conducted to determine whether the differential phase low coherence reflectometer may be used to measure the action potential propagation.

STATUS OF RESEARCH AND PARTNERSHIP:

The UT Austin group has constructed a scanning PSOCT system capable of measuring RNFLT and PR/UD maps in a group of primates with monocular glaucoma. The processing algorithm is being refined to minimize speckle noise in the data and to optimize reproducibility of the measurements. Studies are underway to characterize the registration robustness and variance in sequential images recorded over time and changes in PR/UD maps as a result of glaucoma progression. Our working hypothesis is that a decrease in PR/UD will be an early metric for loss of retinal ganglion cells. The Massachusetts General Hospital group is using a slit lamp interface to their PSOCT instrument to determine a normative human database for the PR/UD maps and then study glaucoma patients for comparison. An algorithm is being developed to discriminate between glaucoma and normal eyes and assess PSOCT measures of glaucoma progression in humans.

ISSUES:

1.Research Focus: The clinical objective to develop an improved imaging modality for screening and diagnosis of glaucoma has remained the principal objective of our research. An important conclusion of our research is that quantitative measurements of phase retardation from scattering tissue samples corrupted with speckle noise is possible and valuable for many clinical applications of PSOCT.

2.Delays caused by technical problems

3.Communication between partners: We are exchanging research results through videoconferencing. We have found regular videoconferences enhance the research efforts at both institutions.

4.Technology transfer to industrial partners: Each group has filed disclosures to respective University intellectual property offices. We have ongoing relationships with Humphrey-Zeiss and Lumenis, Inc.

5.Guidance for clinical trials

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung and Blood Institute (NHLBI)

PROJECT TITLE: Integrated Control of Vascular Pattern Formation

ABSTRACT:
This Bioengineering Research Partnership assembles a team led by two biomedical engineers and a molecular physiologist to focus on the integrative control of vascular pattern formation. While vascular assembly and pattern formation will be needed as critical elements of successful therapeutic collateralization of progressively ischemic organs and in tissue engineering of various tissue substitutes in the future, remarkably little is known of the cells involved, the array of signal molecules and their genetic regulation, and the biophysical factors regulating the spatial and temporal dynamics of vascular pattern formation. Key questions now are: what is the origin of cells responsible for the investment of arterioles with contractile cells and what are the signals that control their proliferation, migration, and differentiation? An integrative systems approach is proposed to measure the dynamics of arteriolar pattern formation in vivo across time scales from the embryo to the adult, and spanning spatial scales from genes to cells to whole networks, and to create a new generation of computational approaches to understand the complex interplay of multiple interacting cells and signal molecules. The specific aims are 1) to determine the role of PDGF and TGF-$ in arteriolar pattern formation during embryonic development, 2) to determine the cell types involved, role of PDGF and TGF-$ signaling, and spatial and temporal patterns of arteriolar assembly in adults, and 3) to develop and use a new cell-based computer simulation to perform integrative spatio-temporal analysis of the arterialization process in the embryo and adult, including multi-signal control of fibroblast and smooth muscle cell proliferation, migration, and differentiation. The multidisciplinary team will utilize unique gene-targeted mice in conjunction with innovative in vivo measurements, and integration of the data into the new computational models will improve understanding of the gene circuitry regulating arteriolar pattern formation. The long term goal is to define the mechanisms that control arteriolar pattern formation, and to provide the basis for powerful therapeutic vascularization procedures that function in the native environment in vivo.

STATUS OF RESEARCH AND PARTNERSHIP:
We have completed development of a transgenic mouse line containing a cre inducible TGFb receptor II dominant negative gene. Of major significance, we observed <1/2 the expected frequency of double transgenic mice containing TGFb RII DN transgenes in two founder lines. These results are extremely exciting in that they provide the first direct evidence demonstrating that TGFb signaling in SMC is required for normal development. Another critical unresolved question in the field is whether pre-existing SMC, circulating SMC progenitor cells and/or local

mesenchymal cells give rise to SMC within newly formed arterioles during vascular remodeling. We are performing remodeling experiments in normal mice that have been lethally irradiated and had a bone marrow transplant with cells from our SM MHC cre x floxed ROSA LacZ mice to permit assessment as to whether bone marrow derived cells contribute to arteriolar remodeling. If we see LacZ + cells that have invested new vessels it will provide unequivocal evidence that bone marrow derived cells contribute to arteriolarization and undergo full differentiation into SMC. In the guided arterialization studies, we showed that sequential delivery of angiopoeitin-1 after VEGF stimulation produced normal hierarchical patterning of small arterioles, while VEGF alone did not. In the computational automata modeling study of arterialization guided by growth factors or hemodynamic stresses, we were able to predict both new capillary development lengths and arteriolar development quantitatively. This is the first in silico multicellular model to capture effects of multiple growth factors and cell types in coordinated remodeling of the vascular network.

ISSUES:

Some operational issues in the partnership have been identified as important to the teamwork involved in the study. We found that regular joint lab meetings were very important, because new results in one aspect of the team, for example a new cell investment result in a hypoxia model, could affect work being done by another member of the team quite rapidly. Another example was team assessment of how to handle local microvessel inhomogeneity of myosin heavy chain promoter penetration, and this was critical to define the project direction of several of the involved students and post-docs. The new stem cell projects arose in a very positive and rapid way out of team meetings on this subject area. In our case, the face to face exchanges of ideas and examination of data has been essential. One reason is that the team is addressing issues ranging from tissue level questions and computer modeling to molecular genetic regulation, and the cross-training of students and young scientists has been very effective with the local mix of people bringing different perspectives to the same problems.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB)

PROJECT TITLE: 7 TMR (GPCR) Drug Discovery, Microfluidics & HT Flow Cytometry

ABSTRACT:
High throughput (HT) screening is integral to drug discovery. While flow cytometry is known for its ability to measure cell responses, its power in the homogeneous analysis of ligand binding or molecular assembly and its potential for high throughput are not well-recognized. The possibility of displaying virtually any molecule in a format compatible with particle-based analysis as well as the novel approach of plug-flow flow cytometry for sampling times ~1 sec could make flow cytometry a powerful alternative for the real-time analysis of molecular interactions. Thus, we propose four projects that bring together expertise in bioengineering and biomaterials, receptors and cell biology, and flow cytometry instrumentation. The first two projects concern biomaterials. In the first project, we propose to express the proteins relevant to signal transduction and termination (seven trans-membrane receptors – 7TMR, receptor tails, G protein sub-units, arrestins, and receptor kinases) in forms appropriate for flow cytometry. These proteins will have epitope tags suitable for homogeneous attachment to beads as well as fluorescent groups suitable for detection by conventional flow cytometry. In the second project, we will employ biomaterial display and detection strategies compatible with flow cytometric analysis. Beads will be used as platforms to display the molecules, to analyze molecular assemblies, to examine enzymatic activities, and to examine inhibition by combinatorial drug libraries. Projects 3 and 4 will involve instrumentation development, fluidics, micro-machines, and automation. In the third project, we will develop fluid handling approaches for cells and beads. We will target throughput rates of 1 sample per second, or near the industrial standard of 100,000 samples per day, using commercial fluid handling components for the types of assays described in Projects 1 and 2. In the fourth project, we will develop and implement micro-fluidic sample handling approaches compatible with flow cytometry using novel elastomer-based micromachine technology. We have set a goal of 10 samples per second or 864,000 samples per day, exceeding the industrial throughput standard by nearly an order of magnitude. By integrating bioengineering, biomaterial, molecular, cellular and flow cytometric expertise, we expect to develop test platforms for high throughput analysis of molecular interactions with commercial potential in drug development. The resulting

technological advances will allow us at the same time to define mechanistic details of cell activation through GPCR mediated pathways.

STATUS OF RESEARCH AND PARTNERSHIP:
The UNM team meets monthly and the project teams meet weekly. In the biological arena, we have established assays for high throughput flow cytometry that include: cell-based assays for GPCR and integrin ligand binding using fluorescent ligands; cell-based assays for GPCR initiated cell responses such as intracellular calcium elevation; cell-based adhesion assays for cells in suspensions; bead-based assays for GPCR molecular assemblies involving intracellular components; bead-based assays for GPCR tail peptide assemblies and phosphorylation; liposome/bead assays of transmembrane transport; and generalized bead-based approaches to analyze protein complexes. We have attacked a problem identified as key to drug discovery – HT single step discrimination of agonists, antagonists, and partial agonists for GPCR, the target of 50% of current prescription medicines. In the technological arena, we have described instrumentation (Patent Pending, HyperCytTM) that approaches a rate of 100 samples/minute with <1% particle carryover from well to well. This approach uses air bubbles to separate ml sized samples with low carryover. We have developed a mathematical description of carryover. We have developed on-line microfluidic mixing strategies, including “wavy boundaries” for submicroliter samples and anticipate sampling rates up to 20 samples per minute. We have coupled HyperCyt to high speed sorting. Together, these approaches provide new opportunities for low cost, small volume, high throughput screening of cell and bead-based molecular target assays at rates approaching 100,000 assays per day, and with multiplexing, up to 1,000,000 assays per day, promises to make flow cytometry a competitive screening tool in research and commercial settings.

ISSUES:
Our experience has shown that team projects are most effective when at least one engineer, one biomedical scientist and one trainee are partnered. Of more than 30 inventions and patents, about half required these teams. We have found that there are some cultural differences that separate biomedical scientists and engineers, and that providing lunch to the whole team once a month is a good way to bring the group together.

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung and Blood Institute (NHLBI)

PROJECT TITLE: MagScrew TAH Testing thru Preclinical Readiness

ABSTRACT:
The fundamental goal of the proposed program is to bring to the point of clinical readiness a new, electrically powered, totally implantable TAH, based on the MagScrew actuator and the biolized blood pump. The specific aims to meet this goal are: (1) To design and develop an advanced technology, fail safe, electronic control unit (ECU), which will maintain the patient 's life after an electrical failure, until maintenance is performed. The ECU also contains hardware and patient monitoring capability, and a telemetry function. (2) To build and test refined versions of the remaining system components, based on current state of the art technology. (3) To integrate the components into a functional, complete system. (4) To perform in-vivo performance tests, exercising system capabilities. (5) To perform in-vivo durability tests. (6) To perform bench endurance tests. (7) To complete this work incompliance with FDA Design Controls Regulations.
As a consequence of this design and testing effort, surgeons will have another, superior choice among relatively limited TAH alternatives. The "biolized "pump of the MagScrew TAH has pericardial valves combined with biological, protein blood contacting surfaces, and a long track record of extremely rare thrombo-embolic episodes in calves, despite the absence of anti-coagulation. In addition, the MagScrew actuator is the conceptually simplest and most rugged of those available for TAHs, with very few contacting or rubbing surfaces. Mechanical failures have very few possible sources, which clearly increases both reliability and long-term durability. While the clinical need for TAHs is consistently estimated to be much smaller than that for VADs, it is of a size both nationally and internationally to be of commercial significance. In the United States, it may exceed $1B per year in potential sales. The TAH market will support several suppliers, if not as many as now pursuing the VAD market. To those patients who will need a TAH, the potentially very limited supply of alternatives is of literally life and death significance.

STATUS OF RESEARCH AND PARTNERSHIP:
The actuator and blood pump assembly is built, has been extensively bench tested, and is entering its in-vivo test program. A breadboard ECU is used to run the ongoing tests, and the fully implantable unit is being constructed. The TETS has passed through several prototypes, based on developing an optimal integration with the system. The implantable and wearable batteries will shortly be delivered by WGL. The bread boarded full system has had preliminary testing at FMT. The development of the implanted wiring harness is lagging. This is a critical component with

respect to system reliability, and specifying a design to meet all requirements has gone slowly. Fully implantable system calf demonstration implants are planned in the next six months.

The partnership is working smoothly, with extensive use of meetings, teleconferences, and the computer to enhance communication. The relationship between the CCF and FMT has continued to expand from the base of the TAH, and both WGL and WB will have future roles in these new projects

ISSUES:
Time and sufficient hardware (money) to do all of the things that must be completed to bring the TAH to fruition are always issues. Communication is also a factor that must always be considered. The system design is tightly integrated, and it is very difficult for any one partner to make a decision without information from another partner. We have to watch this area every day.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB)

PROJECT TITLE: Biomedical Applications of Electroative Polymers

ABSTRACT:
The objective of the Bioengineering Research Partnership program is to refine materials and establish methods for application of electroactive polymers in prosthetics and interventional medical devices. The electroactive materials of interest to us are those that undergo substantial shape change when exposed to an electric field. They are attractive as actuators because of their high energy density – the amount of energy that can be imparted to a load for a given volume or mass of active material, the magnitude of the strain response to an applied field, and their flexibility and toughness when compared with more common electroactive ceramics. Both "found" materials and materials developed expressly for electromechanical activity have been shown exhibit strains of five to 50 percent or more and elastic energy densities of one Joule per cc or more.

Two target application areas have been chosen: (1) next-generation prosthetic blood pumps for treatment of end-stage heart disease, and (2) robotic manipulators for minimally invasive surgery, particularly for use in confined spaces such as the thorax. These disparate applications share the need for very compact, efficient and uncomplicated means of actuation. Both suffer today from the need for bulky actuation mechanisms that must remain physically distinct from the parts which pump blood or manipulate tissue. The technology to be developed under this program will blur the lines between structure and actuator, leading to modes of therapy that are not currently available.

The Materials Research partner is working to optimize electroactive polymers for use in the target medical devices, and develop methods for fabrication of the required multilaminate actuators. As these materials are fundamentally different from the active materials of actuating mechanisms used by engineers in the past, the Mechanical Engineering partner is working to develop new design methodologies. The Bioengineering partner is developing prototype devices to demonstrate the potential of the technology and lay the ground work for full development of new devices. Device development is staged so that simpler, proof-of-concept designs are built first, followed by more sophisticated designs as materials and design tools are developed.

STATUS OF RESEARCH AND PARTNERSHIP:

Materials development work in the past year has focused on the development of new dielectric elastomers. This class of electroactive polymer is promising because of favorable mechanical characteristics and relative ease of processing. Most materials used to date are silicones or polyacrylate that provide high energy density due to their dielectric strength. We are developing high dielectric constant materials which we expect to provide similar energy densities at lower electric fields. These are being formulated as insulating polymer matrix-dielectric enhancer aggregates. The effects of aggregate composition, particle size and distribution on both dielectric and mechanical properties are being studied.

The mechanical engineering partner has focused upon mechanical characterization of finished materials and development of both analytical and finite element models, also with an emphasis in this period on dielectric elastomers. Models have been developed for circular thin film membrane and annulus geometries.

The bioengineering partner has concentrated upon testing of proof-of-concept prototypes and investigation of different forms of actuators that will take best advantage of material properties and processing requirements. This year, we investigated changes pressure-volume characterization of circular dielectric elastomer membranes with activation, providing data for use in future design work and for validation of the analytical models. Four to one changes in diaphragm compliance with activation have been demonstrated. We also began development of techniques for assembling multilayer diaphragms for operation at higher pressures.

ISSUES:
The partnership is operating effectively. Whole-group meetings, one-on-one meetings and electronic communications among partners are all quite effective; we rely most upon electronic communications. Effective partners are motivated chiefly by the desire to work on new problems in a collaborative area. Joint funding enables them to devote the necessary time to the work.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Allergy and Infectious Diseases (NIAID)

PROJECT TITLE: Dynamic Properties of Bacterial Adhesins

ABSTRACT:
The main goal of the proposal is to develop a comprehensive structural picture of how mechanical force affects the functional state of microbial adhesins. Specific adhesive proteins enable bacteria to recognize ligands leading to the adhesion and colonization of various living hosts or environmental niches, and finally infection. Whereas bacterial adhesion to tissues or to medical implants often occurs in the presence of mechanical force generated by shear-flow of body fluids, the effect of drugs such as adhesion inhibitors are typically tested ex-vivo under static conditions. A growing number of experimental observations that mechanical forces acting on adhesins may modulate the affinity and selectivity of adhesins to their ligands should give rise to major concern among bioengineers and in the medical profession. If the functional states of adhesins are indeed altered by mechanical forces, the ex-vivo testbeds that are currently employed by these communities do not adequately simulate the in-vivo conditions under which bacterial adhesion and infection of host cells or to implant surfaces occurs. Indeed, soluble inhibitors that prevent adhesion in static conditions have been shown to be ineffective in preventing adhesion in the presence of shear stress.
In order to test the extent to which mechanical forces may alter the structure and thus the functional states of adhesins, we propose to characterize the dynamic properties of the most common type of bacterial adhesin - FimH – which is a lectin-like adhesive subunit of type 1 (mannose-sensitive) fimbria of enterobacteria and vibrio. In the course of our preliminary studies we have identified distinct structural variants of the Escherichia coli FimH adhesin where shear-flow can induce their preferential binding to target cells, possibly by switching their specificity between the mono-mannoside (1M)* and tri-mannoside (3M) receptors. We have also conducted steered molecular dynamics (SMD) simulations in which tension is applied between the receptor-binding residues and the C-terminal end of the FimH lectin domain to develop structural hypotheses how mechanical forces acting on the binding site may affect the tertiary structure of FimH. Some of these hypothesis has been tested experimentally and proven to be correct. Part of the studies describing the effect of mechanical forces on the FimH function has recently been published (Thomas et al, 2002, Cell).
Deriving a comprehensive understanding of the structure-function relationship of adhesins under static and dynamic conditions requires that molecular biology tools are employed in concert with x-ray crystallography and novel powerful nanoanalytical tools to probe, characterize and simulate non-equilibrium protein structures as they relate to function. This can best be approached by a team of investigators with complementary expertise from various departments. E. Sokurenko is a medical microbiologist specialized in the pathogenesis-related, structure/function, evolutionary

and epidemiological studies of bacterial adhesins. V. Vogel is a bioengineer specialized on the development of nanoanalytical tools for studying molecular assemblies at interfaces, and recently for deducing the role of mechanical force in regulating the functional states of the cell adhesion protein fibronectin.

STATUS OF RESEARCH AND PARTNERSHIP:

During the first year of the project we demonstrated that FimH-mediated binding of bacteria to surfaces coated with purified receptor compounds demonstrate "catch-bond"-like shear-dependent mechanism. It was found that at a shear of 0.1-0.5 dynes/cm2 the binding bacteria exhibit transient mode of adhesions that was converted to steadily rolling mode or firm adhesion at shears above 1 dynes/cm2. Shear stress above 10 dynes/cm2 inhibited the initial attachment of bacteria. However, under this level of shear the binding established at lower ones was further enhanced by causing surface-rolling bacteria to convert rapidly to firmly bound ones. Furthermore, bacteria that were attached under shear of 5 dynes/cm2 could be readily detached if the shear is reduced to 0.1 dynes/cm2. However, the firmly bound bacteria were observed to switch back rolling and detaching more slowly than observed originally at the low shear. In nature, this could allow the bacteria to bind very firmly in the strong pulsate flows typical in vivo in order to remain bound during the low flow periods.

We also have created a cell-free assay that reconstitutes shear activation as seen in whole bacteria by using bacteria-derived purified fimbria and monomannosylated bovine serum albumin. Attachment of the purified fimbria to erythrocytes and to mono-mannose covered beads switched from loose to firm upon a 10-fold increase in the shear stress applied. These results show that purified fimbrial structures by itself act both as a nanoscale force sensor and receptor-binding switch.

ISSUES:

The main conclusions of our studies accomplished during the first nine month of funding are that FimH adhesin interaction with the purified monomannose receptor involves a "catch-bond" -like mechanism. This could be accomplished only by the team effort of molecular microbiologists and bioengineers, thus, showing the importance of combined efforts of investigators from different fields.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB)

PROJECT TITLE: Micro-Instrumentation for Genetic-based Assays

ABSTRACT:
The goal of this research effort is to bring together a multi-disciplinary team (Chemists, Engineers, Life Scientists) to develop integrated microfabricated tools to carry out PCR/LDR (Polymerase Chain Reaction/Ligation Detection Reaction) assays for the detection of cancer diagnostic markers in K-ras oncogenes. Our efforts will focus on the fabrication of instrumentation aimed for clinical diagnostic applications, which will require simple components (transportable) and ease of use without sacrificing detection sensitivity and selectivity, as well as possessing high throughput capabilities. The proposed instrument will have unique but common attributes-namely, a small instrument footprint, minimal manufacturing costs, the ability to mass produce various components, and the capability of performing the assay from sample preparation to detection with fluid handling and detector optics integrated directly on a small platform. In addition, the sub-systems, which will be developed for this application, are modular in design (task-specific chips) so that the individual components can be assembled to carry out complex assays and interchanged for greater flexibility. The devices to be fabricated as part of this research program are:

- Fast thermal cyclers for PCR amplification of DNAs in nanoliter volume chambers with automated sample and reagent delivery.
- Micro-electrophoresis devices machined in polymethylmethacrylate (PMMA) containing multiple separation channels for the high speed processing of ligation products.
- DNA hybridization chips (micro-arrays) fabricated in plastics with nano-fluidic channels for LDR capture. The DNA micro-array substrates will offer robust immobilization chemistries that can tolerate typical thermal and chemical DNA hybridization/denaturation conditions.
- Ultrasensitive near-IR fluorescence instrumentation using solid-state components (diode lasers and detectors), which can be operated in a scanning mode to read multiple electrophoresis channels and/or DNA micro-arrays with extremely high sensitivity.
- Injection molding of miniaturized plastic devices for maximizing production rates and minimizing fabrication costs.
- Materials characterization of the unique substrates that will be used for the micro-electrophoresis and hybridization-based assays. Also, new bonding procedures will be investigated to aid in device assembly.

- Micro-pumps for nanoliter per minute volume flowrates in microchannels used for fluid handling in pressure driven systems.

The core technology development is based upon our well-equipped micromachining facilities located in the Center for Advanced Microstructures and Devices (LSU-CAMD), which contains a synchrotron X-ray source and a multi-million dollar micromachining facility. The multi-disciplinary nature of the research team we have assembled for this Bioengineering Research Organization is well suited for attacking specific issues associated with the diverse and involved scope of this research project. Our goal will be to develop an aggressive multi-disciplinary research program for micro-instrumentation development at LSU. Using the existing resources already located at LSU, our research team will be able to develop integrated devices using novel fabrication techniques. We are one of the few facilities that have expertise in LIGA processing, which allows the fabrication of microstructures with extremely high aspect ratios and over several scales (nm - mm).

STATUS OF RESEARCH AND PARTNERSHIP:

As a result of this BRP project, we have developed several polymer-based devices for performing PCR, assembling microarrays, modifying polymer surfaces for attaching capture probes, microelectrophoresis, and on-chip real-time molecular diagnostics using single molecule detection. To date, we have published over 24 research papers emanating from our efforts in this BRP in peer-reviewed publications. Of these papers, all have been published with at least two authors being participants of this BRP.

We have supported the work of 4 post-doctoral fellows, 8 Ph.D. candidates, 4 Master's students, and 3 undergraduate students. In addition, many of our students are classified as belonging to underrepresented groups in Science and Engineering. One of our Ph.D. candidates of this BRP is currently on the faculty of Mississippi State University (female, African-American).

From the efforts of our BRP, we have established a Center for Bio-Modular Microsystems (CBM2), which has won an in-state competition to represent Louisiana as the research component in their EPSCoR grant. In addition, CBM2 has submitted an NSF STC application.

ISSUES:

None.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Arthritis and Musculoskeletal Diseases (NIAMS)

PROJECT TITLE: Bioengineering Research Partnership-- Muscular Dystrophy

ABSTRACT:
In a recent submission to AJP:Cell Physiology, Cells on Gels: Adhesion vs Differentiation of Skeletal Myocytes, we show that substrate flexibility is central to controllable growth of single myotubes. Although differentiated myocyte cultures have been studied for years, the syncytia they form are generally highly branched, interconnected, and complex. In order to grow isolated, well-defined myotubes and study adhesive mechanisms, collagen strips were μ-stamped and crosslinked to polyacrylamide gels with a tunable elastic modulus. Cell differentiation on stiff gels (but not rigid substrates) was prominent at 2-4 weeks with notable striation accompanying fusion; cells on 10-fold softer gels did not express the differentiation markers nearly as strongly or as organized. In contrast, adhesion strength as measured by micro-peeling of cells, showed that myocyte adhesion increases monotonically with substrate stiffness. Myogenesis thus reflects an optimal balance of contractility and adhesiveness as influenced by substrate compliance. The novel peeling method can be used both to visualize the real-time dynamics of adhesions (eg. GFP markers), as well as to compare the adhesion characteristics of normal versus dystrophic muscle cells.

In another recent submission to BIOPHYS J, Pathway shifts and thermal softening in temperature-coupled forced unfolding of spectrin domains, we report temperature-dependent single molecule AFM results on helical bundle spectrin repeats typical of those found in dystrophin. We show that as T approaches Tm, mean unfolding forces decrease; and circular dichroism studies demonstrate a nearly proportional decrease of helical content in solution. The results also imply a thermal softening of a helical linker often existing between repeats which otherwise propagates a cooperative helix-coil transition to adjacent repeats. The very low unfolding forces imply that domain unfolding may be part of dystrophin's physiological function.

In our recent paper, Muscle-specific expression of insulin-like growth factor I counters muscle decline in mdx mice (J CELL BIOL 2002), we show promising regeneration results. Because insulin-like growth factor 1 (IGF-1) has been shown to enhance muscle regeneration and protein synthetic pathways, we asked whether high levels of muscle-specific expression of IGF-1 in mdx muscle could preserve muscle function in the diseased state. In transgenic mdx mice expressing mIgf-1 (mdx:mIgf(+/+)),muscle mass increased by >40% leading to similar increases in force generation in extensor digitorum longus muscles compared with those from mdx mice. These and added results suggest that a combination of promoting muscle regenerative capacity and

preventing muscle necrosis could be an effective treatment for secondary symptoms caused by a primary loss of dystrophin.

We find that dystrophic lesions in both murine models of limb-girdle and Duchenne MD have increased proton transverse relaxation time (T2), an apparent proton diffusion constant, and elevated dynamic contrast enhancement. Changes in these fundamental parameters result in MRI tissue contrast on T2 and diffusion weighted images following contrast agent administration. Imaging and spectroscopy is now being performed at the Advanced Magnetic Resonance Imaging and Spectroscopy facility at U.Fla. in association with the National High Field Magnet Lab, making use of some of the highest field and most sensitive magnets in the world.

STATUS OF RESEARCH AND PARTNERSHIP:
Fine.

ISSUES:
None.

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung and Blood Institute (NHLBI)

PROJECT TITLE: Biology, Biomechanics and Atherosclerosis

ABSTRACT:
Cardiovascular disease remains the number one cause of death in the United States today. In addition to a plethora of genetic and environmental factors, the diet and lifestyle of Western populations continues to have a profound negative impact on the prevalence of atherosclerotic disease. Significant advances have been made in the management and treatment of the clinical sequalae of this disease process. However, many of these treatments are geared towards the management of catastrophic clinical events, and our appreciation of the basic pathophysiology of atherosclerosis is still lacking in terms of our understanding of the fundamental biology and biomechanics of the atherosclerotic lesion.

Intense efforts have been made over the past decade to identify the factors responsible for the initiation, development, and rupture or erosion of atherosclerotic lesions. Several central theses have emerged from these studies. Mechanical factors impinging on the arterial wall are of major pathophysiologic importance throughout the atherosclerotic disease process. This includes both the effects of fluid shear stress as well as physical stress within the arterial wall and atherosclerotic lesions. Second, inflammatory mechanisms involving the production of reactive oxygen species, expression of pro-inflammatory gene products, apoptosis, and mononuclear infiltration of the arterial wall appear to be central pathogenic mechanisms in all stages of atherogenesis. What is lacking is a comprehensive understanding of the potential convergence of fluid shear forces and physical stresses within the arterial wall and the combinatorial impact of these forces on inflammatory responses within the arterial wall.

Our Bioengineering Research Partnership (BRP) is dedicated to establishing a consortium of investigators from Emory University School of Medicine and The Georgia Institute of Technology devoted to obtaining a greater understanding of the biology and engineering of this fundamental problem of great clinical importance. This BRP expands upon established collaborations to incorporate expertise in basic vascular biology, imaging technologies, fluid mechanics, arterial wall mechanics, cardiac surgery, and interventional cardiology. A key feature of the BRP is that we are making use of explanted human hearts obtained from cardiac transplant recipients to provide a unique model system to study living, human coronary arteries with established atherosclerosis. The in vivo studies are augmented by a series of cell culture studies designed to explicitly examine the effects of defined mechanical forces on inflammatory responses and apoptosis in a controlled setting. Our Aims are:

I. Determine the Distribution of Stress and Strain in Atherosclerotic Plaques in Relation to Markers of Inflammation and Apoptosis
II. Evaluate the Inflammatory Response Characteristics of Vascular Smooth Muscle Cells to Defined Levels of Mechanical Strain
III. To Determine the Detailed Hemodynamic Environment of Atherosclerotic Lesions in Coronary Arteries of Explanted Human Hearts.
IV.Examine the Responses of the Endothelium to the Flow Dynamics Found in the Micro-Environment of the Atherosclerotic Plaque.

STATUS OF RESEARCH AND PARTNERSHIP:
We are just coming to the end of the first year of our partnership. Thus far, a major focus of the modeling studies has been improved imaging of the explanted human coronaries and registration with subsequent histology. We are currently using MR, microCT and ultrasound modalities to image isolated segments of the coronaries. It has become clear to us, that these modalities are complementary in nature that all must be developed in parallel. The fluid mechanics group worked on developing and validating our computational methods. They employed MR phase contrast methodology to measure inflow into the aorta at the aortic root and outflow through the descending aorta. The wall mechanics group has developed a scheme to imbed arteries in radio-opaque media (MMA plus barium sulfate). This is a crucial step needed to implement our scheme for three dimensional reconstruction of plaque morphology from histological sections. It enables us to correct for shrinkage and sectioning artifact and also provides a means for registration (orientation) of the sections. The endothelial biology group has recently identified bone morphogenic protein-4 as a potential mediator of oscillatory shear-induced monocyte adhesion. Finally, the smooth muscle biology group has studied the effects of NO as an inhibitor inflammatory responses. They have shown that NO can suppress strain-mediated pro-inflammatory responses thus identifying a novel mechanism through which localized areas of endothelial dysfunction can cause local, pro-atherosclerotic events.

ISSUES:
No major scientific issues have evolved during the first year of the partnersip. Our only difficulties have arisen with the management of funds between institutions.

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute (NHLBI)

PROJECT TITLE: Design of Biocompatible NiTi Surfaces

ABSTRACT:
The biocompatibility of Nitinol, a group of shape memory, superelastic alloys equally suitable for orthopedics, dental applications, vascular and organ surgery has been challenged due to its high Ni content (~54 % by weight). Ni is known as a toxic and allergenic, though essential to the human body, element. A first glance at Nitinol surfaces revealed that they are depleted of Ni and formed basically from Ti oxides. This observation was accepted as a rule delaying systematic studies of Nitinol surface. It is, now, known that Nitinol surfaces can be either of Ti or Ni-nature depending on treatment. Thereby, their biocompatibility can vary tremendously. The corrosion resistance of Nitinol, vital for long-term performance of implants, also exhibited inconsistent behavior both in vitro and in vivo. The nature of this inconsistency had not been explored before this study. Passivity of Ti-based surface oxides was considered as the only factor responsible for the Nitinol corrosion performance. The goal of this two-year project was to explore regularities in Nitinol surface formation, to design biocompatible, highly corrosion resistant surfaces employing simple, cost effective chemical and electrochemical procedures, to characterize their chemistry, structure, and stability, and to evaluate their comparative biological performance in vitro.

STATUS OF RESEARCH AND PARTNERSHIP:
Surface.The most significant achievements are: development of smooth and rough amorphous Nitinol surfaces with low Ni content; proof of the adverse effect of ethylene oxide sterilization on Nitinol surface chemistry; demonstration of the fact that ground surfaces with residual deformation are prone to chemical heterogeneity upon heating even at low temperatures and the discovery of Ni accumulation on the surface upon heat treatments of the material.

Corrosion. Polarization potentiodynamic and potentiostatic corrosion tests as well as scratch tests were performed in as-received and treated states to evaluate the localized corrosion resistance and scratch healing ability of the material. The comparative corrosion performance of sand blasted, smooth-drawn wires, as well as Nitinol wires with original black oxide resulting from the processing procedure was analyzed. The role of intermetallics in the corrosion performance of Nitinol defined.

Biocompatibility.Three biological studies were planned to evaluate the response of cells to designed surfaces. Study on human peripheral blood lymphocyte proliferation, HPBL, is almost completed. Study, on the response of human vascular endothelial cells (HMVEC), is in progress. The third study, on the factors affecting blood compatibility, will be performed during third year (extension without additional funding)when all biological data will be analyzed and prepared for publication.

ISSUES:
Partnership proved to be very productive. Collaboration with the Memry Corp. and partners from the biomedical field is very intensive. Since our multidisciplinary project is very short time-wise and large in essence, the collaborators have not had the chance to meet all together. This, however, has not affected the performance. Results of the study were presented at three conferences. Four out of six submitted articles have already been published. Three more must be prepared.

There was a delay in obtaining NiTi alloy from the company as well difficulties in hiring an electrochemist. In the mean time the work with commercial Nitinol wires was initiated. The budget was tight. Ames Laboratory and the Metal Preparation Center helped tremendously providing free access to the equipment for sample preparation and surface analysis that employs state-of-art techniques.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Neurological Disorders and Stroke (NINDS) and National Institute of Biomedical Imaging and Bioengineering (NIBIB)

PROJECT TITLE: Intracortical Visual Prosthesis

ABSTRACT:
The development of an implantable human cortical visual prosthesis has been a goal of neuroprosthesis research for 30 years. During this time, the NIH has funded intramural and extramural studies to advance fundamental technologies and address biological questions necessary for the design and fabrication of an implantable system to stimulate the primary visual cortex with intracortical microelectrodes. Although previous work addressed portions of these issues, the focus has primarily been on technology, and fundamental questions remain in three critical areas of research:

Physiology: How can we maximize the amount of information transferred to the primate brain through an array of intracortical stimulating electrodes? In particular, what is the optimal manner of delivering stimulus through the electrodes, and how can stimulation through multiple channels be patterned to best control perception?

Electrode Technology: Can intracortical electrodes be designed, fabricated, and implanted, allowing for long-term safe chronic stimulation of the primate visual cortex by large numbers of electrodes?

Implantable Stimulation Hardware: Can reliable modular implantable electronic packages, capable of driving large numbers of electrodes, via transcutaneous RF power and bi-directional data links, and suitable for surgical implantation, be designed and fabricated?

The overall objectives of the Intracortical Visual Prosthesis BRP are to advance the technology sufficiently to provide a reasonable expectation of reliability and safety for implantable hardware, and to develop an animal model to perform crucial psychophysical and electrical stimulation studies. This 4-year project will culminate in an analysis of data from the fundamental electrical stimulation and psychophysical studies of an animal model, and the development of a completely implantable multichannel stimulation system, including chronically implantable stimulation electrodes. Our long-term goal is to develop an implantable system that will provide usable

vision for a large population of persons with blindness. The goals of this 4-year project are to answer the fundamental questions, above. Our short-term goals are to: 1. Develop a primate animal model for testing the sensory responses to large numbers of parallel intracortical stimulation electrodes. 2. Extend earlier human work on point-phosphene perception to a more general approach that tries to exploit other V1 tuning properties, such as orientation selectivity, to create a richer visual feature set. This will be done by a combination of recording and stimulation techniques in highly trained monkeys performing psychophysical tasks. 3. Demonstrate safety, efficacy, and electrochemical stability of our proposed intracortical electrode arrays using a combination of in vitro and in vivo testing. 4. Determine the optimal physical configuration for, and design a high-reliability implantable inductively-powered cortical stimulator, interfaced to an external computer controller. 5. Develop safe implantation methods, including pre- and post-operative imaging techniques, to optimize and minimize the duration of implant surgical procedures.

STATUS OF RESEARCH AND PARTNERSHIP:
All partners have participated in a series of team meetings during the first 9 months of this project. Inter-institutional contracts are in place. Preliminary animal studies have been performed to access the feasibility of implanting large numbers of intracortical electrodes in area V1 of the primate cortex. Key animal psychophysical studies have been demonstrated. Several conference papers and posters have been presented. One journal article has been accepted, with another journal article in preparation. During this first year of the BRP we have prepared for a series of multi-electrode implantations in a non-human primate animal model. The participation of the team members and the group dynamics are excellent.

ISSUES:
Presently there are two key issues that our team is agressively devising studies to address: Electrode design and consistency, and the design/development of an animal model to demonstrate the feasibility of integrating multiple artificial visual percepts.

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung, and Blood Institute (NHLBI)

PROJECT TITLE: High Frequency Nonlinear Acoustic Intravascular Imaging

ABSTRACT:
Intravascular ultrasound (IVUS) imaging is a technology that permits tomographic visualization of a cross section through the vessel wall. Its development has provided a powerful new method to assess plaque morphology in vivo. However, while new catheter designs are markedly improved on their predecessors, image quality has not seen significant gains due to the primitive nature of the ultrasound transducer designs. Increasing the frequency of the transducer above the current state of the art 40MHz holds the potential to improve image quality, although higher frequencies are attenuated rapidly in biological media and the depth of penetration is therefore reduced. One possible method of enhancing the quality of IVUS images may be to exploit the effect of nonlinear propagation (harmonic imaging) of the ultrasound signal as it passes through the tissue. Despite the fact that harmonic imaging is now becoming a standard modality in the latest commercial B-mode ultrasound scanners with a frequency range up to 4.0 MHz, there is no evidence of attempts to develop a harmonic imaging system for significantly higher frequencies, which would be suitable for intravascular applications. In this application we propose to investigate the generation of tissue harmonics at high fundamental frequencies (20 to 40MHz) suitable for intravascular application. This will be pioneering work in the field of medical acoustics. The major driving force for our project will be clinical necessity. We envisage that the implementation of high frequency harmonic imaging will dramatically improve image quality and allow better delineation of plaque geometry and composition. High frequency ultrasound transducers will be designed and built comprising traditional ceramic materials and novel polymeric devices fabricated using MEMS technology. Finally advanced signal processing methods will be designed and developed to accurately predict plaque composition from high frequency nonlinear acoustic data.

STATUS OF RESEARCH AND PARTNERSHIP:
Specific Aim 2 has consumed the bulk of our activity. We originally proposed to characterize the transducers using a hydrophone, however we have been unable to locate one which is capable of operating at high-frequency and wide bandwidth. We therefore designed a series of experiments using in vitro phantoms to provide information on the beam pattern. These phantoms comprise a 1mm ball bearing, a wire phantom, and a wire grid phantom. Dr Hazony fabricated the unfocussed ceramic transducers that will act as our "gold-standard" for comparison with the broadband MEMS transducers. One of the long term goals of this project was to characterize

custom-engineered PVDF transducers produced using MEMS technology. However, prototypes of this transducer have been made available to our group within a few months of funding, and we have concentrated our efforts on assessing the beam pattern produced from these novel devices. These data show that the center frequency of the device is 32 MHz and it has a 110% bandwidth. We have manufactured these transducers with f-numbers of 2.0, 2.5, and 3.5 and are in the process of characterizing their beams. These transducers will also be compared to the Boston-Scientific Atlantis transducer. Finally, we have recently started to use our transducer to image tissue. Human aorta was obtained at autopsy and affixed to a motorized stage with 6 degrees of freedom. The tissue was imaged with the f-2.0 transducer and the sample sent for histological preparation.

ISSUES:
None.

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GRANTING NIH INSTITUTE/CENTER: National Cancer Institute (NCI) and National Institute of Biomedical Imaging and Bioengineering (NIBIB)

PROJECT TITLE: Advanced Multi-Spectral Imaging (MSI) System for Medical Diagnostics

ABSTRACT:
The goal of this project will be to develop a novel multi-spectral imaging (MSI) system using the synchronous luminescence (SL) concept to rapidly detect cancer in-vivo. The proposal will address the problem of real-time in-vivo identification and characterization of malignant and pre-malignant tissues in the upper gastro-intestinal (GI) tract. While presence of Barrett's mucosa is simple to detect endoscopically, at the present time dysplasia and early cancer is found only by extensive biopsies. The typical protocol is four quadrant biopsies at 2-cm intervals of the Barrett's mucosa. While this is the standard technique, it only provides 3-5 % sampling of the mucosal surface where dysplasia and diffuse cancer may be found. The remaining 97-95% of the mucosa is not sampled.
Laser-induced fluorescence (LIF) spectroscopy has already been used to detect cancer and high-grade dysplasia in Barrett's esophagus. However, that system uses a contact technique, which samples a 1 mm area of tissue at each measurement. While the contact LIF system is better than the pinch biopsy technique, a new system is needed to allow examination of the entire surface of the mucosa. To address this important need in imaging, we will develop a real time synchronous imaging system based on state-of-the-art acousto-optic tunable filter (AOTF) technology coupled to an endoscope.
A unique MSI technology using the SL technique will be developed to obtain spatially resolved images of the slight differences in luminescent properties of malignant versus non-malignant tumors. This will provide a faster and more accurate in-vivo analysis without biopsy. The unique imaging aspect of this MSI system will provide real-time spatial information, allowing for comprehensive diagnosis of large areas of interest.
An interdisciplinary approach will be used to perform the proposed research to provide results in an efficient and cost effective manner. We will be working in close collaboration with the University of Tennessee (UT) School of Veterinary Medicine, and medical researchers with expertise in clinical studies at the Thompson Cancer Survival Center (TCSC). Following development of this technology, initial studies will be performed on two model systems, biopsied tissues as well as laboratory animals at Oak Ridge National Laboratory and UT. Once the system has been optimized, clinical in-vivo studies will be performed on human subjects at the TCSC in Knoxville, Tennessee.

STATUS OF RESEARCH AND PARTNERSHIP:

During this reporting period, we have made important progress in several aspects of the project. We have completed the setting-up of a multispectral imaging (MSI) laboratory system. This system employs laser excitation, an imaging fiber probe system for signal collection, an acousto-optic tunable filter (AOTF) for wavelength selection, and a charge-coupled device (CCD) for detection. We have successfully tested the MSI system to image tumors in laboratory mice. By using a tunable optical parametric oscillator (OPO) laser system, we are capable of tuning our excitation light over the spectral range of 405- 700 nm. We have successfully evaluated the MSI system using fluorescence measurements with nude mice. Two series of experiments were performed. First we used normal nude mice provided by Dr. E. Michaud at ORNL to optimize the optical set-up of our MSI system. In the second measurement series, we used mice with tumors, which were provided by Dr. Robert Lee from Ohio State University. Male athymic mice were purchased from Charles River Laboratories (Wilmington, Massachusetts). To generate tumor xenografts, human oral carcinoma cells were injected subcutaneously into the mice. In this study, we investigated tumors in mice that have been injected with an exogenous fluorophore (e.g., porphyrin) used as tumor indicator. Exogenous fluorophores have been developed for many different purposes and applications in the field of biological monitoring, ranging from the monitoring of cellular function using fluorescent reporter dyes or molecules for various biochemical species to the demarcation of tumors with a relatively recent class of exogenous fluorophores. Using both the MSI system, we have successfully performed spectral imaging measurements of tumors on mice that have been injected with porphyrin.

We have also set up an LIF device for spectral measurements using an OPO laser for excitation. This instrumental setup has several advantages over our previous fixed-excitation LIF system since the new system incorporates the capability for rapid variations of excitation wavelength and the possibility for time-resolved measurements using an intensified charge-coupled device (ICCD). We have conducted measurements to optimize the time gate and delay parameters in order to obtain maximum signals with minimum interference from the laser scatter. Following these optimization procedures, we have performed endoscopic time-resolved LIF measurements at various excitation wavelengths ranging from 415-460nm on a canine laboratory animal using the OPO-LIF system at the University of Tennessee- Knoxville (UTK). LIF spectra were collected on various sites such as esophagus, stomach, upper and lower GI of the canine animal. Our system showed excellent performance producing LIF spectra with high signal-to-noise values. Various known biomarkers such as porphyrin, riboflavin and FAD present in the tissues were clearly detected. The fluorescence profiles of normal tissue in the canine GI tract exhibited spectral profiles similar to those we have recorded with normal tissues. The results of this evaluation indicated that the OPO-LIF system is safe for future clinical tests.

We are working closely with our partners, the Thomson Cancer Survival Center (TCSC) and the University of Tennessee- Knoxville (UTK) in this project. The animal study protocol we have submitted last year has been approved by the UTK College of Veterinary Medicine and ORNL. This has allowed us to perform an evaluation test of our instrumental system using canine animals at UTK. The protocol for clinical studies has also been approved by the Internal Review Boards (IRBs) at both institutions (TCSC, ORNL).

ISSUES:

N/A. We are currently proceeding well in the system evaluation phase of the project.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB)

PROJECT TITLE: Long Wavelength Quantum Dot-based probes – cell tracking

ABSTRACT:
We will develop new technologies to employ quantum dots in biological imaging in vitro. In an evolving program, quantum dot technology will be developed specifically for (1) cell identification and tracking of cells in tissues, (2) tracking cell proliferation through multiple generations in tissues (3) vasculature location, and (4) deep 3-D imaging of Qdot-labeled polymer scaffold structures relative to the Qdot-labeled cells in engineered tissue models.
Quantum Dots Corporation will prepare new types of Qdots that have properties suited to deep imaging of cells and structures in tissues. Particular emphasis will be placed on near infrared Qdots for imaging deeper in tissues. The Science and Technology Center at Carnegie Mellon University will then develop methods to derivatize Qdots for existing and new applications in cell biology. The STC will initially use available Qdots and conjugates, label cells by a variety of means, then test the cells for fluorescent brightness, stability of labeling, and cell survival and function. As newer quantum dots become available, they will be similarly tested and compared with existing Qdots and existing organic fluorescent probes, e.g., Alexa dyes and cyanine dyes. Fluorescence lifetime imaging capability will be added to the 3-D grating imager in the STC for enhancing signal-to-background in deep tissue imaging by taking advantage of the relatively long emission lifetime of Qdots.
To obtain feedback for the development program we have included collaboration with the recently formed Bone Tissue Engineering Center at Carnegie Mellon University. In this collaboration we will examine the utility of the developing Qdot technology for studying cell location, movement and proliferation in the 3-D structures of engineered bone tissue. This is a particularly challenging and relevant system that requires Qdot technology to extend cell tracking to denser and more highly scattering tissue matrices, including hydroxyapatite-containing artificial bone matrices. By the conclusion of this project, we expect to have instrumentation and probes to perform time-resolved multicolor imaging at millimeter depth in many natural and artificial tissues. Thus the technology will be generic and have utility in many biological and medical applications.

STATUS OF RESEARCH AND PARTNERSHIP:
Our partnership began in 2001, and the research project was funded in April of 2002. Quantum Dot Corporation has been an effective partner in both producing quantum dots and in devising new surface coatings for use in biology. During the past year, we have used several different surface coatings to stabilize quantum dots for use in aqueous solution. By modifing these

surfaces, we have (1) greatly increased spontaneous cellular uptake of Qdots in vitro, (2) labeled engineered tissue matrices, (3) increased circulating lifetime in vivo, and (4) coupled quantum dots to biological materials for labeling and targeting. Our coated quantum dots retain their fluorescence in vivo for at least two months. Emission wavelengths of our quantum dots have progressed to the near infrared (~750nm), although the IR-emitting quantum dots are less stable than the far-red emitters (days rather than months.

ISSUES:
We have no significant issues in our partnership. Personal visits, emails, and weekly phone conferences have ensured that communication and exchange of materials have been easy and effective.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Neurological Disorder and Stroke (NINDS) and National Institute of Biomedical Imaging and Bioengineering (NIBIB)

PROJECT TITLE: High Resolution SPECT/CT Imaging of Systemic AA-Amyloidosis in Mice

ABSTRACT:
High resolution imaging is becoming an invaluable tool in biomedical research much as it has to the clinician. In the clinic, imaging offers a precise, non-invasive means of diagnosis and directly influences both the therapeutic approach and prognosis. Unfortunately, the development of high-resolution imaging tools demanded by researchers has lagged behind that of the clinic; thus, characterization of the kinetics of in vivo pathology and the subsequent development of novel, effective therapeutics has been hampered. This is particularly true in the field of amyloid-related diseases which include Alzheimer’s disease, type II diabetes and primary (AL) amyloidosis. It is impossible to fully appreciate and understand the complexity of these diseases, and the means by which they may be halted, without the ability to perform longitudinal studies in individual animals in vivo. To that end, the development high-resolution micro-imaging technologies capable of detecting and quantifying amyloid deposits in vivo is warranted and imperative. We intend to address these important issues through the design and application of a powerful new dual-modality imaging technology, microSPECT, combined with microCT, supported by state-of-the-art 3-D image reconstruction and analysis software. This new technology will be employed to identify radiolabeled amyloid deposits in live animals and present the amyloid distribution within the context of a high-resolution CT image of the visceral terrain. With this technology, the goal of quantifying organ-specific amyloid burden in vivo is attainable. The goals are thus to: (i) Complete the design and implementation of a high-resolution, small-animal specific dual SPECT/CT imaging system. (ii). Develop a system of amyloid quantification in which microSPECT image data can be directly correlated to amyloid burden. (iii) Use these technologies to study the progression of systemic AA-amyloidosis in two murine models and the regression thereof in response to novel immunotherapies. This study will not only result in technological advancements in the field of small-animal imaging and amyloid-specific radio-tracers but will also provide a wealth of information on the natural progression of amyloidosis in vivo and establish a paradigm for the screening of therapeutic drugs in animal models of human disease. Furthermore, the translation of amyloid-specific imaging technologies will yield tangible clinical benefit.

STATUS OF RESEARCH AND PARTNERSHIP:
During the initial 9 mos. of our amyloid imaging program we have achieved several of our first year milestones. At present, we are evaluating the efficacy of radio-labeled amyloidophilic

monoclonal antibodies (mAb) in a murine model of localized light chain (AL) amyloidosis. Radiochemistry: Our intended goals for the project were to radiolabel antibodies and other imaging agents to be used for microSPECT/microCT analysis of amyloid deposition in mice. We have radioiodinated the mAb 11-1F4 and its F(ab')2 fragments with I-125 and measured their biodistribution and micro-distribution in mice bearing sub-cutaneous AL amyloidomas or systemic (AA) amyloid deposits. Established techniques including chloramine T radioiodination, micro-autoradiography and standard biodistribution studies have been very informative and clearly discern the reaction of the mAbs in vivo. In the short term we intend to expand these mAb studies and evaluate a more durable radioiodination protocol (using a succinimidyl-iodobenzoate linker) as well as test radioiodinated serum amyloid P-component as a standard for imaging amyloid. At present we are using two independent machines to gather microCT and microSPECT data, but plan to engineer both detectors upon a single gantry within the next 6 mos. We have devised a system which allows us to accurately co-register the data from each modality using I-125 containing capillaries. Reconstruction and Software: For CT imaging, we have completed Feldkamp conebeam code including support algorithms for focus of attention. In addition, the first version of parallelized iterative code will be completed by end of year one. For the SPECT data sets, we have implemented the first version of iterative maximum-likelihood code for use with a parallel-hole collimator. We have also developed a 3-D segmentation system for delineating organ boundaries by defining regions of interest in 2-D microCT datasets, which represents our initial steps in developing a quantitative amyloid imaging system. By the end of summer, a network-based file repository system will be brought on-line to facilitate sharing of raw scanner data, reconstructed CT and SPECT images, as well as image analysis results and other data. In the next several months we will perform a study designed to evaluate the effect of x-irradiation dosage on various facets of murine hematopoiesis and physiology. This is a joint study performed at ORNL and the University of Tennessee College of Veterinary Medicine that will determine the effects of x-ray exposure consistent with the protocol for collecting serial microCT data in living mice.

ISSUES:
There were problems with budget approval for the National Lab sub-contract which delayed access to their budget for 4 mos. Otherwise, we have not encountered any major problems.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB), National Institute of General Medical Sciences (NIGMS), and National Cancer Institute (NCI)

PROJECT TITLE: Development of Q-dots as biological probes

ABSTRACT:
The long-term goal of this Bioengineering Research Partnerships is to develop semiconductor nanocrystals fluorescent probes (Q-dots) technology that will provide biomedical research with better tools for diagnosis of diseases and biomedical techniques and instrumentation necessary for basic research of cellular and molecular structure and fundamental life processes. This includes Q-dot probe synthesis, bio-conjugation techniques, dedicated optical instrumentation and unique imaging methodologies. We will develop optimized protocols for Q-dot synthesis with desired optical, physical and chemical properties. Various spectroscopic and structural measurements will be used to fully characterize Q-dots. This information will be fed back into the synthesis for optimization of the desired properties. Bio-conjugation schemes and labeling protocols will be developed for biomolecules in fixed and living cells.

STATUS OF RESEARCH AND PARTNERSHIP:
We have continued developing peptide coating technology for q-dots that yields excellent colloidal and photophysical properties. This coating allows to directly bioactivate q-dots without a need for bioconjugation. We also developed a simple one-step approach to functionalize CdSE/ZnS nanocrystals with DNA and used it in DNA microarrays technologies and Fluorescence in-situ hybridization (FISH) of combed DNA molecules. Peptide-coated (q-dots-biotin-peg) probes were targeted to living HeLa cells over-expressing CD14 receptors (part of the glycosyl-phophatidyl-inositol - GPI anchored proteins family) fused with an avidin. This chimeric CD14-avidin protein is a very useful model system for studying the dynamics of lipid anchored receptors in the cytoplasmic membrane of living cells as well as their trafficking and recycling. Movies of the recycling processes of CD14 receptors in living HeLa cells could easily be recorded taking advantage of the high photostability of the q-dots probes. We are currently analyzing the diffusion and directed motion patterns of single CD14-Av-biotin–peg-q-dots in different parts of the cell in order to elucidate their dynamic behavior. These studies will provide better understanding of trafficking and recycling of GPI anchored proteins. We are exploring the use of q-dots for non-invasive, non-radionuclide, molecular imaging in small animals. As a first step we have tried to find out if q-dots are imageable in living mice. Small quantities of red CdTe or CdSe/ZnS q-dots were injected subcutaneously in a nude mouse and the mouse was then imaged using a cooled CCD camera. We were able to clearly locate the site of q-dot injection. We

have conducted toxicity studies in cell cultures and in live animals. Both peptide-coated and polymer-coated q-dots were found to be non-toxic. To improve the sensitivity and deep tissue penetration in these live animal imaging experiments, we are developing NIR q-dots (emission in the range of 700 to 950nm).

ISSUES:
After the PI moved to UCLA, it became clear that the change in research environment will require restructuring of the partnership. The funding for several UCB partners was discontinued and new members (UCSF, UCLA) joined the partnership instead. The research direction was adjusted with exciting new biomedical imaging. In particular, applications to cancer biology and tumor imaging are now vigorously pursued.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Biomedical Imaging and Bioengineering (NIBIB)

PROJECT TITLE: Data display to detect, diagnose and treat critical events in anesthesia

ABSTRACT:
Currently, projects are in progress in anesthesia and ICU patient monitoring. A single display evaluation study is reported as an example for the group's work.
Evaluating a drug display. Monitors that show intravenous drug and effect concentrations currently do not exist. However, using real-time displays of intravenous anesthetic concentrations and effects could significantly enhance intraoperative clinical decision-making and patient safety. Pharmacological models are available to estimate drug concentrations in the brain, and to predict the drug's associated physiological effects. We developed a graphic display incorporating these models to show the predicted present and future concentrations and effects of anesthetic drugs in real-time. That anesthesiologists' benefit from modeled drug concentrations when dealing with simple procedures was shown in earlier work. This study examined the question of how anesthesiologists deal with changes in complexity when using modeled drug information. In the current study, complexity was manipulated by the surgeon who asked for changes in the surgical procedure, requiring immediate changes in the anesthesia plan. As a consequence, the anesthesiologist was required to change drug dosages because of a changing pain level.
Subjects. Thirty-one anesthesiologists with a range of clinical experience participated in this study. We determined three levels of expertise: novice anesthesiologists (CA-1 and CA-2), intermediate anesthesiologists (CA-3 and chief residents) and experts (faculty at the University of Utah Medical School).
Procedure. Subjects were instructed to take care of a simulated patient during surgery. A standardized training familiarized participants in the use of the drug display, the simulator, and the drug delivery equipment (syringe reader, intravenous pumps). The high fidelity METI patient simulator at the University of Utah was used to conduct the study. The drug display (Figure) was placed beside a standard anesthesia monitor. As drugs were administered, pharmacokinetic (PK) drug models predicted effect site concentrations, and pharmacodynamic (PD) models predicted the patient's level of analgesia.
Scenario. The surgery involved shoulder arthroscopy and a non-scheduled (i.e., unpredictable) Bankart procedure on a 62 y/o, 80 kg male. Initial values for the patient were a mean arterial blood pressure of 90, and a heart rate of 65. The arthroscopy was expected to take 20 minutes.
Measures. Performance in analgesia management was assessed by comparing the pharmacodynamic predicted level of analgesia with the level of surgical stimulation (calculated by RMSE). Because the simulator was calibrated such that the cardiovascular responses match the pharmacodynamic predictions, subjects in both conditions had the same cardiovascular information available. Heart rate (HR) and mean arterial blood pressure (MAB) were used to measure periods when the patient responded adversely to pain. The time from skin incision to awakening (spontaneous respiration and eye opening) was recorded. Upon completion of the scenario, subjects answered a workload questionnaire (NASA-TLX).
RESULTS

Painful Bankhart procedure. During the complex, painful Bankhart procedure, differences in cardiovascular stability were due to the drug display. Anesthesiologists in the drug display condition showed higher performance as indicated by MAB and HR being closer to the initial values, and lower pain levels than the control group. Analyzing the patient's heart rate revealed a main effect of condition ($F(1,25)=4.9;p<.05$), with higher heart rate when subjects used the drug display. MBP. Analyzing the mean arterial blood pressure showed that subjects in the drug display condition kept the blood pressure higher than the control group ($F(1,25)=4.4;p<.05$). Pain. The level of pain control showed a similar pattern. We found a reliable difference between the display condition and the control condition ($F(1,25)=5.5;p<.05$), with lower levels of pain in the drug display group.

End of sedation. No expertise difference was found for rapidness of sedation or emergence. However, we observed an effect of the display condition on performance: when using the drug display, control of sedation better matched the patient's requirements (Table 2). Analyzing the time until eye opening showed a main effect of condition ($F(1,29)= 4.3;p<.05$). Patients treated in the drug display condition woke up more than two minutes earlier. Analyzing the time for the respiratory rate to reach 50% of its initial level showed a main effect of condition ($F(1,29)=11.4;p<.01$) with patients reaching this rate more than three minutes faster when treated using the drug display.

Overall time for the procedure. To assess the effectiveness of the delivery of anesthesia we compared the overall time of the procedure for both conditions. The ANOVA revealed a main effect of condition ($F(1,30)=4.8$; $p<.05$), with anesthesia being delivered more efficient when anesthesiologists were using the drug display.

Perceived workload. Using an ANOVA with the factors condition and expertise, the analyses revealed a reliable difference between conditions in terms of subjective performance ($F(1,30)=4.44;p=.045$). Subjects using the drug display rated their subjective performance higher (5.9(1.6)) than the control group (4.5(2.0)).

DISCUSSION

The study examined how a newly developed drug display affects anesthesiologists' performance when facing an unexpected complex task and how the drug display's presence interacts with anesthesiologists' levels of expertise. At the point where the complexity of the scenario increases, we found differences between the drug display group and the control group, with better performance in controlling vital signs and pain in the drug display group, as indicated by the smallest deviations from the initial values and lowest pain levels. Interestingly, no differences were found based on participant's level of expertise. This indicates that the drug display seems to have more of an effect than level of expertise. The means for vital signs and pain level indicate that the novices in the drug display condition actually performed almost as well as the experts in the control group, providing evidence that visualizing pharmacokinetics and pharmacodynamics improves performance dramatically such that a novice can perform at the same levels as an expert. Finally, the duration of the procedure was shorter when subjects were using the drug display indicating that they were not only more efficient when complexity of the procedure increased, but that in being more efficient they needed less time to finish the delivery of anesthesia.

Taken together the results of this study show that an anesthesiologist's performance in delivering anesthesia can be significantly improved by providing modeled data about the pharmacokinetics and pharmacodynamics of the drugs delivered. As a consequence, better control of the patient's vital signs and faster awakening from anesthesia can be observed. The results of the present study are encouraging; they demonstrate that patient safety can be improved by providing anesthesiologists with intraoperative drug information which is currently unavailable.

STATUS OF RESEARCH AND PARTNERSHIP:

This year our partnership grew with the addition of three bioengineers, two anesthesiologists and an ICU director. These investigators support our new activities in physiologic modeling and new applications of the technology in the intensive care unit.

ISSUES:

There are no issues.

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GRANTING NIH INSTITUTE/CENTER: National Center for Research Resources (NCRR)

PROJECT TITLE: Cold Neutrons for Biology and Technology

ABSTRACT:
The Cold Neutrons for Biology and Technology (CNBT) partnership consists of investigators from six universities, the National Institute of Standards and Technology (NIST), Los Alamos National Laboratory (LANL), and the NIH committed to the development of advanced neutron scattering instruments for studies of membrane systems at the NIST Center for Neutron Research (NCNR). Specifically, these instruments will be devoted to basic and applied studies of membranes and
macromolecules in membranes, and to membrane-based technologies that include studies of protein complexes with relevance to bioengineering. The instruments, consisting of a fully dedicated biological advanced neutron diffractometer/reflectometer (AND/R) and a 30-meter small-angle neutron spectrometer (SANS) dedicated 10% to biology, will provide combined advantages and capabilities not currently available in the United States. During the first two years of the project, the AND/R, which has already been designed with the aid of a planning grant from the NSF, will be constructed and commissioned and an existing world-class SANS instrument will be optimized for membrane research. At the same time, a high-performance computer system will be put in place to support the concerted use of neutron diffraction and molecular dynamics methods in order to deduce 3-D structural information from 1- or 2-D diffraction data. Finally, new laboratory space adjacent to the neutron instrument hall will be renovated and equipped to serve the special needs of the partnership and the other biological users. Concomitantly, research and technical staff will be recruited.
The development of the new membrane-optimized instruments will be driven by distinct experiments inspired by the research programs of the CNBT team. The expertise of the team members, drawn from departments of chemistry, chemical engineering, physiology, cell biology, and physics, includes membrane diffraction, small angle neutron scattering, membrane molecular dynamics (MD), biosensors, and biomaterials. Linking neutron diffraction measurements to MD simulations of biomolecular structure is an important objective of the team. We foresee a future when computer simulations will allow three-dimensional detail to be inferred routinely from 1- and 2-dimensional neutron and X-ray data.

STATUS OF RESEARCH AND PARTNERSHIP:
The project is in its second year. We are presently working on six major tasks in order to install and commission the AND/R instrument and to attain the goals of the CNBT partnership. (1) The director for the AND/R has been recruited and Johns Hopkins University has been added as a CNBT partner. (2) A memorandum of understanding (MOU) between NIST and UCI to enable construction of the AND/R using grant funds has been set up. (3) The AND/R is scheduled to be

installed in the experiment hall this summer. It will undergo a series of shakedown experiments during the summer and early fall as part of its commissioning. (4) High-performance computers have been purchased and set up at NIST and at UCI. (5) A new humidity chamber for the measurement of biological membranes has been designed for the SANS instrument. It will be tested this summer. (6) The new laboratory space has been designed and procurements of the furniture and equipment have begun. The partnership is generally in fine shape.

ISSUES:
Nothing serious at present.

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GRANTING NIH INSTITUTE/CENTER: National Cancer Institute (NCI)

PROJECT TITLE: Engineered Antibody EGFR Antagonist Cancer Therapeutics

ABSTRACT:
The objective of this BRP is to develop basic scientific understanding of aberrant signaling by epidermal growth factor receptor (EGFR) in cancer, and integrate this knowledge with development of new mechanism-based EGFR antagonists that will serve as therapeutic lead molecules. The partnership incorporates expertise and approaches from protein engineering (Wittrup), quantitative cell biology (Lauffenburger), computational biophysics (Tidor), and structural biology (Kuriyan). The importance of EGFR as a target in cancer has been validated recently by favorable clinical trial results for inhibitors against both its intracellular (Iressa, AstraZeneca) and extracellular (Erbitux, ImClone) domains.

STATUS OF RESEARCH AND PARTNERSHIP:
1. Antibody Engineering. We have isolated 15 antibody fragments from a human antibody library that bind to domains 3 and 4 of the EGFR. In the coming year, we will affinity mature these antibodies and explore their functional effects on signaling and proliferation in tumor cell lines. We have developed an epitope mapping method for determining which portion of EGFR are bound by a given antibody.
2. EGFR cell biology. Efforts have commenced to examine the cross-signaling between EGFR and the ErbB2 coreceptor that is amplified in many cancer cells. Antibodies will be screened to determine whether cross-signaling can be selectively blocked. Several cell lines will be used to study the effects of new antibodies developed in this project: a human mammary epithelial cell line (HMEC) with varying levels of ErbB2 expression driven by retroviral transfection; and A431 human tumor cells which massively overexpress EGFR and exhibit autocrine cell growth kinetics.
3. Computational antibody design. Computational approaches for antibody design are being developed using a model system for which high resolution structural information is available, the D1.3/hen egg lysozyme pair. Affinity-improving mutations will be predicted and tested experimentally.
4. EGFR structural biology. In order to develop mutants of EGFR amenable to structural and biophysical characterization, we have screened yeast surface-displayed libraries of mutant receptors, and isolated variants with enhanced thermal stability, improved immunoreactivity with

commercial antibodies against EGFR, and reduced structural heterogeneity as assayed by electrophoresis. Crystallization trials for these mutant receptor ectodomains will commence shortly.

ISSUES:
No untoward issues have been raised to date; the partnership is progressing well.

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GRANTING NIH INSTITUTE/CENTER: National Institute of Neurological Disorder and Stroke (NINDS) and National Institute of Biomedical Imaging and Bioengineering (NIBIB)

PROJECT TITLE: GENERAL PURPOSE BRAIN-COMPUTER INTERFACE (BCI) SYSTEM

ABSTRACT:
Signals from the brain can provide a new communication channel - a brain-computer interface (BCI) - for those with severe neuromuscular disorders such as amyotrophic lateral sclerosis (ALS), brainstem stroke, cerebral palsy, and spinal cord injury. BCI technology can allow people who are completely paralyzed, or "locked in," to express wishes to caregivers, use word processing programs, access the Internet, or even operate neuroprostheses.
Up to now, BCI research has demonstrated that a variety of different methods using different brain signals, signal analyses, and operating formats can convey a person's commands to a computer. Future progress that moves from this demonstration stage to practical applications of long-term value to those with motor disabilities requires a flexible general purpose BCI system that can incorporate, compare, and (if indicated) combine these different methods, and can support generation of standard protocols for the clinical application of this new communication and control technology. The development and clinical validation of a general purpose BCI system is the goal of this Bioengineering Research Partnership (BRP) application.
The investigators in this partnership have been in the forefront of research into current BCI methods, and together they have extensive experience in the development of BCI systems. The aims of this proposal are: (1) to develop a flexible general purpose BCI system that can incorporate any of the relevant signals, analyses, and operating formats and can be configured for laboratory or clinical needs; (2) to use the system to compare, contrast, and combine relevant brain signals and signal processing options during BCI operation and thereby develop a standard protocol for applying BCI technology to the needs of individual users; (3) to apply the system and protocol to address specific communication needs of people with severe motor disabilities and show that BCI technology is both useful to and actually used by these individuals; (4) to apply the system and protocol to develop the use of neuronal activity or field potentials recorded within or on the cortex for communication and control, and to define the relationships between these signals and scalp-recorded signals that might be used to guide or supplement invasive methods.

Achievement of these aims and dissemination of the resulting technology to other research groups should advance BCI research from its current stage of laboratory demonstrations to development and validation of a general purpose BCI communication and control technology that can incorporate all relevant brain signals and has clear practical value for those with motor disabilities.

STATUS OF RESEARCH AND PARTNERSHIP:

The BRP's first 8 months have been productive:

(1) BCI-2000, the general purpose BCI system, now has signal acquisition and processing modules for sensorimotor rhythms, slow cortical potentials, P300 potentials, and rhythms recorded from the cortical surface, and application modules for controlling multi-dimensional cursor movements and for operating several simple word processing programs.

(2) The Albany and Tuebingen partners are implementing a comprehensive screening and training protocol that uses a fully portable BCI-2000 system. The protocol is being applied in an initial set of people with ALS in their homes.

(3) A BCI-2000 based study in Albany is showing that people can use scalp-recorded sensorimotor rhythms to control two-dimensional cursor movements with speed and accuracy comparable to that reported in monkey studies that use neuronal activity recorded within cortex. The Atlanta partner is adapting the protocol to a robotic arm. These studies are extending the possible applications of non-invasive BCIs to include neuroprostheses, motorized wheelchairs, and other robotic devices.

(4) The Albany partner is working with investigators at Washington University to use BCI-2000 for device control with signals from arrays placed on the cortex before epilepsy surgery. Both actual movements and movement imagery are associated with sharply focused changes in sensorimotor rhythms. With brief training, people can use these rhythms to control cursor movements.

(5) BCI-2000 with documentation is being given to other research labs. About 15 labs have requested it, and many are using it for BCI studies and for other research as well.

ISSUES:

Work is going extremely well. The most critical issues concern how to allot the Partnership's time and effort among the many exciting opportunities now presenting themselves.

In addition to the active clinical collaboration between Tuebingen and Albany, we are exploring additional clinical efforts, with the ALS clinic at Drexel University in Philadelphia and with the BRP consultant Dr. Donchin in Tampa. In addition to the active collaboration between Albany and Washington University in evaluating cortical surface signals, we are exploring development of comparable studies with several other major university centers. These possibilities are very exciting and could be highly productive. At the same time, they would make substantial demands on BRP personnel.

A growing number of research groups are requesting BCI-2000. These groups vary greatly in their expertise. Some are able to use BCI-2000 with minimal consultation, while others need much help. Providing this help can be inordinately time-consuming. We are currently trying to define reasonable criteria for providing this help and reasonable limitations on the time and effort we will allow it to occupy. We are also receiving requests from people wanting to come to the Wadsworth Center to learn to use BCI-2000. It will probably be most efficient and effective to develop a several-week course to be given periodically, perhaps once a year. This will be a considerable undertaking and will place further demands on Partnership personnel. An application for additional funding to cover this purpose may be indicated.

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GRANTING NIH INSTITUTE/CENTER: National Heart, Lung and Blood Institute (NHLBI)

PROJECT TITLE: Understanding/Improving Fontan Flow Surgeries

ABSTRACT:
Congenital heart defects occur in 1% of live births with 20% of these defects corresponding to complex congenital lesions with only one effective pumping chamber; the latter are termed single-ventricle heart defects. Thus, 2 babies out of every 1,000 will be born with a single-ventricle heart defects. Surgical treatment consists of bypassing the right side of the heart and connecting the systemic and pulmonary circulations in series with the single-ventricular pump. Patients usually need to have a series of surgical treatments, and patients who survive require lifelong, intense medical attention. Cardiologists report that these patients represent 20% of their caseload but require over 50% of their time; this underscores the gravity of problems patients have as well as the need for improvements in existing treatment methods. The current surgical procedure of choice for patients with a single ventricle is the total cavopulmonary connection (TCPC).

The central objective for this multi-institutional BRP project is that development of pre-operative computer-based surgical design methods will advance the state-of-the-art in treating single-ventricle patients and improve their quality of life. The BRP consists of a team of biomedical engineers, pediatric cardiologists and surgeons. Clinical expertise guides project developments, recruits and studies patients, and, most importantly, implements discovered improvements. Engineering tools such as computational fluid dynamics, rapid prototyping, and digital particle image velocimetry provide valuable insight into the fluid dynamics of the TCPC that will direct the development of planning tools. Coordinated in vitro, computational, and in vivo animal and patient studies are being conducted at four institutions: Georgia Tech (GIT), University of North Carolina (UNC), Children's Hospital of Philadelphia (CHOP), and Egelston Hospital of Emory University and Children's Healthcare of Atlanta (CHOA). In vitro experiments in simulated models of TCPC geometries under physiologic conditions along with MRI image processing studies are being conducted at GIT. Computer modeling is being performed at GIT and UNC. In vivo animal studies are being conducted at UNC, and non-invasive 3-D MRI anatomic and flow studies on TCPC patients are being performed at Emory, CHOP and UNC.

The project consists of four specific aims: (1) Qualitative and quantitative assessment of Fontan flow dynamics for different TCPC anatomic geometries and physiologic conditions (for example, rest vs. exercise), to establish optimal TCPC anatomic templates—to be addressed through in

vitro experimental and computational fluid dynamics (CFD), in vivo animal and patient magnetic resonance imaging (MRI) studies. (2) Study the impact of different materials, used in the inferior vena cava (IVC) to right pulmonary artery (RPA) connection (in the TCPC surgery), on the local flow dynamics—CFD and in vivo animal studies. (3) Establish an anatomic and materials database that would be used to validate computationally based designs and surgical planning—CFD, patient MRI, and animal studies. (4) Provide improved designs and surgical planning through the use of pre surgery MRI anatomic information and computational simulations, to optimize the TCPC in an individual patient—patient MRI and computer modeling in conjunction with anatomic templates derived from Aim 1.

STATUS OF RESEARCH AND PARTNERSHIP:
Aims 1 and 2. Computational and experimental fluid dynamics analyses of the anatomic reconstructed geometries are in progress. A rapid prototyping procedure has been developed that successfully makes transparent, solid models of the anatomical reconstruction for digital particle image velocimetry (DPIV). Computational studies also investigated the compliance mismatch in the artery-vein anastomosis in the TCPC. In-vivo animal models of three of the five types of TCPC have been successfully performed.

Aim 3. Progress has been made toward developing the database of patients with different types of Fontan. The patient geometries obtained to date vary dramatically, and this variation reaffirms the importance of geometry in the TCPC connection and demonstrates one of the primary reasons for this BRP. Algorithms to reconstruct the Fontan geometry are a vital component of the integrated CFD modeling and in vitro experiments performed in this study. Significant improvements have been made in reconstructing and segmenting MR images by implementing an automated routine. The automated segmentation algorithm is intensity based but uses a novel “rolling ball” approach that differs from other intensity-based techniques such as region growing and thresholding. The resulting anatomic geometries are smoother and can be obtained with much less user intervention.

Aim 4 work will begin in year 3.

Despite several personnel changes at each of the partner institutions and one of the co-PIs going into private practice, the BRP has functioned well with little impact on the scientific progress.

ISSUES:
The major issue that arose during the first year of the grant was the difficulty that the clinical partners (CHOP, CHOA, and UNC) had recruiting patients for the study. One clinical partner, CHOA renovated their clinical MRI department and this reduced the number of scan slots available. Also at CHOA, 52% of patients screened for study participation were not eligible due to coils that were used after the TCPC surgery to close collateral veins. To remedy this problem, CHOA modified its standard of care to close collaterals with particles that will not interfere with MR scans. As for the other clinical partners, CHOP will hire a study coordinator to increase their patient recruitment, and UNC has just started to recruit patients for year 2. All clinical partners are also increasing their coordination with the surgeons on their staff to make contact with patients regarding study participation soon after the TCPC surgery is performed to increase study participation during year 2.